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CONTRIBUTIONS TO A KNOWLEDGE OF INHERITANCE IN MAMMALS

I. Studies of Color Inheritance and of Linkage in Rabbits.
By W. E. Castle.

II. Fertility and Sterility in the Norway Rat, Mus norvegicus.
By Horace W. Feldman.

III. The Japanese Waltzing Mouse: Its Origin, Heredity and Relation to the Genetic Characters of Other Varieties of Mice. By William Hazen Gates.

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PART I

STUDIES OF COLOR INHERITANCE AND OF LINKAGE IN RABBITS

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With eight plates.

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STUDIES OF COLOR INHERITANCE AND OF LINKAGE IN RABBITS.

CHINCHILLA ALLELOMORPHS OF ALBINISM.

The so-called color-factor of rodents is peculiarly subject to striking variation or mutation. The best-known mutation is the ordinary or complete albino, which develops no pigment at all in its hair, skin or eyes. The ordinary white rabbit (Plate 1, fig. 1) belongs in this category, as do also the white mouse and the white rat. All experimenters are agreed that this type of albinism behaves as a simple recessive character in crosses with the ordinary pigmented type of the same species.

In rabbits there occurs a second type of albino (Plate 1, fig. 2) which has pigmented extremities (nose, ears, feet and tail). This type is known as the Himalayan or Russian rabbit. The two types of albinos are allelomorphs of each other, as I showed in 1905, Himalayan being dominant. Both also are allelomorphs of full pigmentation, a point, the theoretical importance of which, Sturtevant (1913) emphasized.

The ordinary albino guinea-pig is of the Himalayan type, since it has some pigment in its extremities. It is allelomorphic, as Wright (1923) has shown, to all more fully colored types. Of these Wright was able to distinguish four, viz, (1) intense, (2) partially dilute, (3) fully dilute, and (4) red-eyed dilute. Each was found to be recessive to those allelomorphs which preceded it in the series, and dominant to those which followed, the entire series being explicable as the result of variation in a single gene, the several recognizable stages of which, though graded, were entirely stable. In short, the series formed an excellent example of multiple allelomorphs. Considering the series as a whole, it was noted that in the process of pigment reduction through variation in the color gene, yellow pigment was affected first. In the partially dilute stage, yellow became cream; and in the red-eyed dilute stage, yellow was no longer discoverable, though black persisted in considerable amounts. Consequently, in this stage the fur of agouti animals was silver gray in appearance and the retinal pigment was so reduced in amount that the eye gave a red glow by reflected light. When by suitable crosses the gene for yellow coat was combined with that for red-eyed dilution, a variety was obtained in which the coat was white and the eye red.

In 1921, I began a study of the variety of rabbit known as chinchilla (Plate 2, figs. 1 and 2) and discovered very promptly that, not only in appearance but also in genetic behavior, it closely resembles the silver-gray variety of guinea-pig which Wright had studied in my laboratory and named red-eyed dilute. Like the silver-gray guinea-pig, the chinchilla rabbit has white-tipped fur and eyes which are red by reflected light. Likewise in crosses with the yellow variety, white-coated individuals with red eyes are obtained in F_2. Consequently, I have described a chinchilla allelomorph of the color-factor in rabbits as a homologue of red-eyed dilution in guinea-pigs (Castle 1921). There is every reason to believe that the chinchilla mouse represents a parallel mutation in that species (See Schuster 1905 and Feldman 1922).

What I did not realize in 1921, but have since discovered, is the fact that my stock of chinchilla rabbits contained two quantitatively different stages of red-eyed dilution. The two are allelomorphs of each other. The first (Plate 2, fig. 2), which we may for convenience call "dark" chinchilla, produces in a homozygous state, in the first coat, hairs which are faintly but distinctly yellow tipped. The yellow is especially conspicuous in the nape of the neck. In later moults, however, the yellow tinge practically disappears. The second type (Plate 2, fig. 1), which we may call "pale" chinchilla, produces no perceptible yellow in either the first or in subsequent coats. The general pigmentation in this class is much paler. In the nest, the two types are strikingly different and readily separated (Plate 3, fig. 1). At birth only the pigment of the hair-tip is yet visible and that is imbedded in the skin for the most part. The skin-color of the dark type is then similar to that of the fully pigmented gray rabbit, but the skin of the pale type is very much lighter. It contains at this stage, as I should estimate it, not a quarter as much pigment. When the hairs are fully formed, the difference is less noticeable but is still easily recognized even in adult animals. (See Plate 3, fig. 2.)

In 1921, I received from a dealer in England a pair of chinchilla rabbits, none being obtainable at that time in this country. These I mated with each other, but did not succeed in obtaining any living young from them, though both individuals were successfully mated with other animals. My present experimental stock of chinchilla animals has been derived exclusively from the imported male individual. He was mated with females of several different color types, viz, albino, blue, red (yellow), gray, and steel gray. (See tables 1 and 2.) Mated with a gray Flemish Giant doe, he produced 10 gray young. By two steel-gray does (both heterozygous for the "steel" factor, dominant extension), he produced 6 steel-gray and 5 gray young. By a New Zealand Red doe, an intensely pigmented yellow variety, he produced 7 gray young with coats of a reddish-yellow cast, something like that of a Belgian hare but less intense in the shade of yellow. In all matings so far mentioned chinchilla had been completely recessive. It has proved to be something distinct from agouti, extension and yellow. But when the chinchilla male was mated with albino females, only chinchilla young were obtained. This result indicated that chinchilla was of the same nature as albinism, was in fact an allelomorph of albinism and dominant over it. The chinchilla buck, in a mating with a blue doe, known to be heterozygous for albinism, produced three gray young and three chinchillas. This result is in harmony with the interpretation that the albino gametes formed by the doe produced chinchilla young, whereas the remaining gametes produced gray young. It is noteworthy that no blue or blue-gray young were produced, showing that the blue dilution is also a character distinct from chinchilla, as it is from albinism, and that the male chinchilla did not transmit dilution.

One of the albino does (♀ 4463), which was mated with the chinchilla buck, was known from her pedigree to be homozygous for yellow, and heterozygous for dilution. She had 5 chinchilla young, 3 of which were recorded at birth as being "dark, with a tinge of yellow showing on the neck," the other two as "pale, without yellow tinge on the neck." It was supposed, at the time, that dilution probably made the difference between these two classes of young, but this interpretation was later found to be erroneous.

Table 1.—*Matings of original chinchilla male which produced no chinchilla young.*

Mother.	Number and color characters of young.	Record No. of young.
♀ New Zealand Red	7 reddish gray	4891^{a}–7^{a}
♀ Gray Flemish	10 gray	4977–83
♀ Steel Gray Flemish	4 gray, 4 steel-gray	5046–50, 5169^{a}–71^{a}
♀ 4713 Steel Gray	1 gray, 2 steel-gray	5148
Totals:	22 gray, 6 steel gray.	

Table 2.—*Matings of original chinchilla male which produced chinchilla young.*

Mother.	Number and color characters of young.	Record No. of young.
♀ 4463, albino (ccdDee)	3 dark chinchilla, 2 pale chinchilla	4862–6
♀ 4436, blue (Ccdd)	2 gray, 1 steel gray, 1 steel gray chin., 2 pale chin.	4867–70
♀ 3315, Polish albino	1 chinchilla	4931
♀ 4864, dark chin., daughter	2 dark chin., 3 pale chin., 1 chin. ?	5522–7
Total chinchillas:	5 dark, 7 pale, 3 unclassified.	

It seemed, however, to be in harmony with the result obtained about the same time from the blue doe. Two of her chinchilla young were recorded as "pale," the third one being intense. If paleness were due to dilution, then the chinchilla buck might be heterozygous for dilution. But this proved not to have been the case. For when, in F_2, blue-chinchilla young were obtained, they were observed to be quite distinct in appearance from the pale chinchillas. Later events showed that the original chinchilla buck had really transmitted two different types of chinchilla, a dark type and a pale type, each of which has since maintained its distinctness in various genetic combinations. This precludes the possibility that either one is the equivalent of the other plus a detachable modifier. They behave like allelomorphs and may well be regarded as such.

The matter is made clear in the progeny of two daughters of the chinchilla buck by a gray Flemish Giant doe. Both were gray in color like their mother and both were mated with the same albino buck, a white Flemish Giant. (See table 3.) One, ♀ 4980, which had obviously received dark chinchilla from her sire, produced in two litters 13 gray young and 5 chinchillas of the dark type. The other, ♀ 4982, which evidently had received pale chinchilla from her sire, produced 4 gray young and 4 chinchillas, the latter of the pale type. This same mother was mated also with a cinnamon buck heterozygous for the Himalayan type of albinism and produced 8 gray young and two chinchillas of the pale type. These latter, being doubly heterozygous for brown pigmentation and for chinchilla, were used in linkage tests in which they were back-crossed with brown Himalayan mates. All chinchilla young produced in these matings have been of the pale type. Eight of them which have been reared to maturity are clearly such.

By matings between animals carrying the dark type of chinchilla and those which carried the pale type, chinchillas have been obtained which, like the original male, were heterozygotes between the two types. I find that most of the animals which I have selected as most desirable from a fancier's standpoint are such heterozygotes. Perhaps like myself fanciers in the past have unconsciously chosen heterozygotes to breed from, because they came nearest to the type desired, homozygotes of the dark type being too yellow in the baby coat, which is used for fur, and homozygotes of the pale type not being dark enough. If this is true, the case is very similar to that of exhibition Dutch rabbits, which I have elsewhere shown to be heterozygotes between two types, one with too dark a head, the other with too white a body, the heterozygotes being preferable to either homozygous type.

My original chinchilla buck was homozygous for factors A (agouti) and E (extension). In F_1, animals were produced which were heterozygous for one or both of these factors, but this did not materially change their appearance from that of the homozygous sire. In F_2, recessives for both factors were obtained, which are quite different in appearance. The double recessive non-agouti type, aa, I have produced chiefly, if not exclusively, from the pale chinchilla parents. It, of course, lacks the banded hairs and lighter under-surfaces characteristic of agouti animals. Its pigmentation is less heavy than that of an ordinary non-agouti black rabbit, becoming in most parts of the body a dull, faded, brownish-black. But the more intensely pigmented extremities may be described as black. The black pigmented non-agouti chinchilla type under discussion, in accordance with Wright's nomenclature for guinea-pigs, I shall designate "sepia." (See Plate 3, fig. 3.) The corresponding brown-pigmented type is scarcely distinguishable from the ordinary chocolate or "Havana" variety except in its eye-color, which is red. We may designate it red-eyed chocolate. It combines three recessive mutations, non-agouti (a), chocolate (b), and chinchilla (c^{Ch}).

The agouti factor, A, appears to be quite as effective in a single as in a double genetic dose, heterozygotes being indistinguishable from homozygotes among chinchillas, as among ordinary gray rabbits. The same is true of the extension factor, E, in relation to its recessive allelomorph, the yellow mutation, e. Chinchillas of the formula Ee are indistinguishable from those of formula EE. But the double recessive, ee, or "yellow chinchilla" is of a most surprising appearance, since it is neither yellow nor chinchilla and thus presents a double contradiction to its name (Plate 4, fig. 1). As I have stated elsewhere (Castle 1921), its coat is white and its eyes gray. Except for the difference in eye-color, it closely resembles the Vienna-White variety, which has a snow-white coat and blue eyes (Plate 4, fig. 2). Yet the genetic composition of a Vienna white is very different from that of a "yellow chinchilla," for the two varieties, each of which when uncrossed breeds true, since it is a combination of recessive factors, produce gray F_1 offspring when they are crossed with each other. (See Plate 5, fig. 1, and Tables 3 and 4.)

A word may be said concerning the eye-color of the "yellow chinchilla" variety. I have described it as gray. Such is the color of the eye in an ordinary chinchilla rabbit. The eye of an ordinary gray, black, or yellow rabbit is brown in color. I assume that this brown color is due to the association of yellow pigment with black in the iris. A chinchilla rabbit, being incapable (within the limits already indicated) of developing yellow pigment, such pigment is largely wanting in the iris as well as in the coat, so the iris contains only black pigment. In view of the diminished total amount of pigment in the iris of the chinchilla rabbit, the iris color is gray, not black.

The extension factor in rabbits has undergone a dominant as well as a recessive mutation. The latter is well known in the yellow variety. The dominant mutation (E^D) is found in steel-gray and dominant black varieties, such as Black Siberian and some strains of black Dutch. Its somatic effect is greatly to reduce or wholly to obliterate the agouti marking of the fur, which accordingly is black or nearly black (steel gray).

In combination with the chinchilla factor, dominant extension produces either a sepia type (when the agouti marking does not show, Plate 3, fig. 3) or a steel chinchilla type (when the agouti marking shows faintly, as in steel gray rabbits).

Combination of the chinchilla factor with the brown mutation (b) has already been referred to. In the non-agouti form, this combination resembles ordinary chocolate, as already stated. In the agouti form, it becomes a brown chinchilla, differing from cinnamon (brown agouti) in that the agouti banding is not yellow but colorless (white). Steel-brown chinchilla resembles ordinary steel chinchilla, the only difference being that brown pigment replaces black. In all brown-chinchilla combinations, the eyes are redder than in the corresponding black combinations, because brown pigment hides the red-color of the blood in the eye less than black pigment does.

The dilution factor (d) was early in our experiments brought into association with chinchilla in crosses of the original chinchilla male with a blue

TABLE 3.—*Matings of animals, heterozygous for chinchilla, with chinchilla or with albino animals.*

Heterozygous parent.	Chin. or albino parent.	Character of young:								
		Gray.	Steel gray.	Blue gr. or steel-blue gr.	Yel.	Dark chin.	Pale chin.	Steel gr. chin.	Yel. chin. (white)	Unclassified chin.
♀ 4533, Gray	♂ 4863, dark chin.	2	..	1	..	..	..	..	..	1
♀ 4867, Gray	♂ 4869, pale chin.	3	1	2	1	..	..	..	3	1
♀ 4893^{a}, Gray	Do	4	..	..	2	..	3	..	..	..
♀ 4893^{a}, Gray	♂ 4863, dark chin.	3	..	..	..	..	..	..	2	3
♀ 4895^{a}, Gray	Do	6?	..	..	3	..	..	..	2	6?
♀ 4980, Gray	Do	5	..	..	..	2	..	..	..	..
♀ 4980, Gray	♂ albino Flemish	13	..	..	..	5	..	..	..	..
♀ 4982, Gray	♂ 5543, dark chin.	3	..	..	..	..	..	..	..	6
♀ 4982, Gray	♂ albino Flemish	4	..	..	..	..	4	..	..	..
♀ 4982, Gray	♂ 4861, cinnamon	8	..	..	..	..	2	..	..	..
♀ 5049, Steel Gr.	Do	4	1	..	..	..	..	3	..	6
♀ 5805, Gray	♂ 5321, dark chin.	3	..	..	..	..	..	..	..	4
♂ 4977, Gray	♀ Imported chin.	3	..	..	..	..	..	..	..	7
♂ 4977, Gray	♀ 5085 albino Flemish	1	1	..	..	..	..	..	..	8
♂ 4977, Gray	♀ albino	1	2	..	..	..	..	4	..	1
Totals:		63	5	3	6	7	9	7	7	43
		77 fully colored				73 chinchilla				

TABLE 4.—*Other matings involving "yellow chinchilla" (white with gray eyes).*

Mother.	Father.	Young:					
		Gray.	Yellow.	Chin.	Blue Gr. Chin.	Yel. Chin. (white).	Albino.
♀ 4462, albino (ee)	♂ 4863, chin. (Ee)	..	..	0	4	3	2
♀ 4862, chin. (Ee)	Do	..	..	11	3	3	13
♀ 4864, chin. (Ee)	Do	..	..	2	2	2	1
♀ 4864, chin. (Ee)	Do	..	..	6	5	4	1
♀ 4866, chin. (Ee)	Do	..	..	9	5	4	2
♀ 4870, chin. (Ee)	Do	..	..	1	5	2	5
♀ 4542, Gray (Ee)	Do	5	1	..	..	1	..
♀ 4876, Yel. (Cc)	♂ 5519^{a}, yel. chin. (white)	..	4	..	..	..	3
♀ N. Zealand Red	Do.	..	5	..	..	..	..
♀ 5446^{a}, Yel. chin. (white)	♂ Vienna-White	4	..	..	..	..	..

female and with an albino female heterozygous for dilution. In F_2 from these crosses (Table 3) were obtained several blue-gray chinchillas (Plate 3, figs. 1 and 2), which differ from ordinary chinchillas in the same way that blue differs from black, the pigmentation being notably less intense. A blue-gray chinchilla can be distinguished from ordinary blue-gray individuals by the absence of yellow from the coat; for the dilution factor affects yellow no more than it does black, whereas chinchilla does. Accordingly an ordinary blue-gray rabbit has the interior of the ear, the nape of the neck, and the margins of the belly fur of a creamy-yellow color, whereas in a blue-gray chinchilla the corresponding regions are white. So the young of these classes can be distinguished without difficulty even in the nest, when their fur is only a few millimeters long and the eyes are still closed.

GENETICS OF THE VIENNA-WHITE RABBIT.

Wild rabbits, like most other wild mammals, are generally free from white spots except the white or whitish belly and tail. Even these regions are frequently not entirely devoid of color, their more or less complete depigmentation being due to the same genetic factor which produces the gray coat dorsally. When that factor mutates so as to produce a black coat dorsally, then the belly and tail are also black.

But among domestic rabbits, as among other domesticated animals, the coat is frequently white spotted and white spotting is a very definitely inherited character. In general its production depends upon mutation in a single gene, and its inheritance, consequently, is that of a simple Mendelian character, the inheritance ratios being mono-hybrid. Two familiar types of white spotting, differing in the location on the body of the white areas, occur among rabbits. These are known to fanciers as Dutch (Plate 5, figs. 2 and 3) and English (Plate 7, figs. 1 and 2) respectively. The former is an incompletely recessive character in inheritance, the latter a strongly dominant character, yet the two behave in general like allelomorphs in heredity (due to different conditions of the same gene) but it seems more probable that they depend upon two distinct genes very closely linked with each other. Dutch varies through a wide range of grades of whiteness as shown in Plate 6, from a trace of white in the center of the forehead, on the tip of the nose, or on a front foot, to a condition all white except a small spot at the base of the tail. No one, so far as I know, has ever obtained a Dutch rabbit which was all white, the hypothetical grade 18 of Plate 6. A rabbit which appeared to be of this character was figured by Baur (1914, p. 75) which, when crossed with an albino mate, produced a typical Dutch young of grade 6 or 7. Nevertheless, it is now clear that this "blue-eyed white" rabbit, though it produced Dutch-marked young, did not owe its whiteness to the Dutch factor. This rabbit was referred to by Baur under the name by which it is known among fanciers, Vienna-White. The genetics of this variety has since been more fully investigated by Baur's pupil, Endre Pap (1921), who has shown that the all-white coat associated with colored eyes is a simple Mendelian recessive character. This interpretation I am able to confirm from experiments which have been in progress since February 1922. At that time I obtained from a dealer in England a pair of Vienna-White rabbits. They had snow-white coats and blue eyes. Mated with each other they produced a litter of 8 young, all like themselves with white coats and colored eyes. The male (Plate 4, fig. 2) was used extensively in crosses with other varieties. Mated with self-colored (unspotted) females, he regularly produces white-spotted young resembling low grade Dutch of grade 1 to 3 (Plate 5, fig. 1). Occasionally the Dutch pattern is more strongly expressed in the heterozygous young, attaining to grades 4 to 6 of Plate 6, or it may not be expressed at all, the young having no white in the coat (grade 0, Table 5). These less usual conditions of the heterozygotes are probably due to peculiar genetic composition of the self-mother. Thus the grade 0 young of Table 5 were all borne by a family of chocolate rabbits which also gave self-young in crosses with high-grade Dutch rabbits, an unusual result for that type of cross which will be discussed subsequently.

Table 5 classifies the young produced by the Vienna-White sire, when mated with self (unspotted) does, in terms of the grading scale for Dutch rabbits (Plate 6). In this classification, grade 0 means an unspotted (self) rabbit, and grade ¼ means a mere trace of white spotting, such as a star in the forehead, a small spot on the nose, or white toes on a fore foot. The other grades will be sufficiently clear from an examination of Plate 6. It will be observed that more than half of the 143 young classified in Table 5 fall in Dutch grades 1 and 2. This accordingly may be regarded as the normal expression of the Vienna-White character in a heterozygous state.

Table 5.—*Classification in terms of the Dutch pattern (Plate 6) of 143 rabbits heterozygous for Vienna-White, whose mothers were self-colored (unspotted), their father being the original Vienna-White buck (Plate 4, fig. 2).*

Grade	0	¼	1	2	3	4	5	6
No.	7	10	58	25	21	13	5	4

Total: 143

The eye of a Vienna-White rabbit has been characterized as blue, but it is very different in appearance from the eye of a blue-coated rabbit. The eye of the latter is indeed of a slate-blue color similar to the coat; but that of a Vienna-White rabbit is whitish blue, like the blue eyes of a blond man. The resemblance is due to a like cause, absence of pigment from the front wall of the iris. An eye of this type is often seen in coach dogs and piebald horses, where it is known as a "wall-eye." We may use the same designation for the similar eye in rabbits. In rabbits heterozygous for Vienna-White, the wall-eye appearance is frequently seen in certain sectors of the iris only. In general those heterozygotes with most white in the coat show the most extensive development of the whitish iris, as will be seen in Tables 6 and 7. The iris is usually completely white, as in homozygous Vienna-Whites, in those heterozygotes of Dutch grades 4 to 7. It is usually 50 to

75 per cent white in those of grade 2 or 3, but is less than 50 per cent in those of Dutch grade 1 or lower. There is thus seen to be considerable correlation between the degree of development of Dutch pattern in the coat and white iris in the eye.

A similar correlation exists in ordinary Dutch rabbits free from the Vienna-White character, between the whiteness of the coat and that of the iris, for Dutch rabbits show the same kind of "wall-eye" (Plate 5, fig. 3) that is seen in Dutch-marked Vienna-White heterozygotes, but the threshold of its occurrence is higher in ordinary Dutch rabbits than in Vienna-White heterozygotes. Compare Table 8 with Tables 6 and 7. Table 8 is based

TABLE 6.—*Relation between "wall-eye" and Dutch pattern of coat in F_1 rabbits heterozygous for Vienna-White.*

Grade of Dutch.	Percentage of iris affected.					Totals.
	0	25	50	75	100	
0	1	..	1	..	..	2
¼	4	1	1	..	1	7
1	3	9	1	3	1	17
2	1	1	1	2	1	6
3	..	2	1	3	3	9
4	..	..	..	..	3	3
5	..	..	..	1	2	3
Totals:	9	13	5	9	11	47

TABLE 7.—*Relation between "wall-eye" and Dutch pattern of coat in F_2 rabbits known to be heterozygous for Vienna-White.*

Grade of Dutch.	Percentage of iris affected.					Totals.
	0	25	50	75	100	
¼	1	..	..	..	..	1
1	3	2	1	2	..	8
2	..	1	2	3	3	9
3	1	2	1	1	1	6
4	..	..	1	..	1	2
5	..	..	..	1	..	1
7	..	..	..	..	1	1
Totals:	5	5	5	7	6	28

TABLE 8.—*Relation between "wall-eye" and Dutch pattern of coat in Dutch rabbits free from Vienna-White.*

Grade of Dutch.	Percentage of iris affected.					Totals.
	0	25	50	75	100	
2	8	..	..	..	..	8
3	6	..	..	..	..	6
4	9	1	..	..	..	10
5	3	..	..	..	..	3
6	4	1	..	..	..	5
7	..	2	..	..	..	2
8	2	1	1	..	..	4
9	..	1	..	..	1	2
12	..	..	2	1	..	3
14	..	1	..	1	..	2
15	..	..	..	..	5	5
16	..	..	..	1	3	4
Totals:	32	7	3	3	9	54

on an examination of the eyes of 54 ordinary Dutch rabbits ranging in grade from 2 to 16. No true Dutch rabbit of lower grade than 4 was found to have even a trace of wall-eye, but those of grade 9 or higher (that is rabbits whiter than the typical Dutch of the fancier) nearly always have a wall-eye, 50 to 100 per cent white. But Vienna-White heterozygotes frequently develop a "wall-eye," when there is very little white in the coat, or even none at all. (See Table 6.)

It is thus evident that Vienna-White and Dutch have similar effects on the pigmentation of both the eye and the coat of rabbits, but the action of Vienna-White is much more powerful. In a homozygous condition it drives all pigment from the coat and from the front wall of the iris; in a heterozygous condition, it develops a Dutch-marked coat, usually of grade 1 to 3, with an iris 25 to 100 per cent white.

Dutch, on the other hand, whitens the coat more strongly than the eye. It is doubtful whether the "dark" form of Dutch (Plate 5, fig. 2) described by Castle (1919) ever produces a wall-eye, though the whitening of the coat may, in homozygotes, go as far as grade 6. The "white" form of Dutch (Plate 5, fig. 3), when homozygous, expresses itself in a coat pattern of grade 12 to 17, and in a wall-eye 25 to 100 per cent white. When heterozygous, it rarely or never produces a wall-eye, but finds expression in the coat in a Dutch pattern, commonly of grade 1 to 4. Vienna-White heterozygotes with a like amount of white in the coat nearly always show a wall-eye, as is evident from Tables 6 and 7.

An experiment as yet incomplete indicates that White-Dutch and Vienna-White, not only have similar effects in whitening the coat and the iris, but also those which are of the same physiological nature. For Vienna-White may apparently replace one dose of White-Dutch in a rabbit of grade 14. A rabbit of that grade was known by pedigree or shown by test to be heterozygous for both characters, yet somatically it was as white as a homozygous White-Dutch rabbit.

This leads to the inquiry whether Vienna-White may not properly be regarded as a form of Dutch, an *ultra-Dutch* perhaps. The answer to this question is that while in its physiological action Vienna-White is similar to (but not identical with) White-Dutch, the two are shown by linkage studies to be at different genetic loci and in different linkage groups (chromosomes).

The Vienna-White buck was mated with self (unspotted) angora does of various colors—gray, black, yellow and blue. In this way 55 young were obtained all short-haired and Dutch-marked. They are included in the 143 young all sired by the same Vienna-White male and classified in Table 5. These F_1 animals have been mated in various ways with the object of producing an F_2 generation containing double recessive combinations suitable for use in linkage tests. Those tests will be described in another part of this paper.

LINKAGE IN RABBITS.

INTRODUCTION.

In undertaking a comprehensive study of linkage in rabbits, it has been my plan by means of crosses to associate each simple (unifactorial) genetic variation of rabbits with every other simple genetic variation of the species, secure as speedily as possible the double recessive combination from each cross and then back-cross to this the F_1 double heterozygotes. If linkage exists between two genes it should be revealed most speedily and certainly by such back-cross tests. It may seem to the reader that this could have been done quite as effectually and much more rapidly by making a smaller number of crosses, each one of which involved simultaneously several characters. But in reality I have found it rarely practicable to work with more than two character pairs at a time, because relations of epistasis and hypostasis make it impossible to classify many individuals with certainty when a larger number of character pairs is involved.

Leaving out of consideration multiple allelomorphs, of which there are three or possibly four sets, there are in rabbits nine different single-gene characters, whose linkage relations have been investigated. These are:

1. A, a, agouti vs. non-agouti.
2. B, b, black vs. brown.
3. C, c, color vs. albinism.
4. D, d, intensity vs. dilution.
5. Du, du, self vs. Dutch pattern of white spotting (a recessive).
6. E, e, extension vs. restriction (yellow).
7. En, en, English (a dominant white spotting pattern) vs. self.
8. L, l, short vs. long hair.
9. V, v, self vs., Vienna-White.

In the case of all but one of these nine characters, the dominant condition is the original state found in wild rabbits, and the recessive is a novelty or mutation. But in the case of number 7, English pattern is the mutation and is dominant. It is easier to test for linkage between a dominant and a recessive character, than between two recessives, for one of the parent varieties to the cross is in this case usually a double recessive, and F_1 can be crossed back directly to this, without the necessity of producing an F_2 generation. Of the nine characters listed, one (Dutch) may be disregarded for the present since it is either an allelomorph of English or closely linked with it. The more easily made tests, for linkage of English, will serve to show what linkages may be expected also in the case of Dutch.

The possible combinations in pairs which one can make among eight different things (in this case genes) number 28. Table 21 contains a list of the 28 gene relations to be tested for linkage in rabbits and shows what progress has been made in covering the program outlined. Of the total 28, 20 have been tested conclusively, one inconclusively, and 7 tests have not yet been made. But of the missing 7, the probable outcome is in no case very doubtful because of positive results secured from tests already made. Thus when two characters are linked with each other, it is theoretically certain that a character showing linkage with one will also show linkage with the other.

Two linkage systems have been demonstrated. The first involves linkage between the two genes B and C. The second involves linkage between three genes, English, Dutch, and Angora.

If these two groups of linked genes are borne in two chromosomes, as the chromosome theory of heredity would suggest, there remain only four other genes unassigned to linkage groups, namely genes A, D, E, and V. Each of these is probably independent of the others as shown by tests A-D, A-E, D-E and E-V (Table 21) but certain tests with V are yet incomplete. That A is independent of the B-C linkage group is shown by test A-C. That it is independent of the other linkage group is rendered probable by the test A-En, which is being supplemented by other tests. That D is independent of the B-C group is shown by the test C-D, that it is independent of the second group is shown by tests B-C and C-E, that it is independent of the second group is shown by tests E-En and E-L. That V is independent of the B-C group is shown by tests B-V and C-V; that it is independent of the second group is shown by the test En-V; that it is independent of A, D, and E is indicated by experiments as yet incomplete.

Accordingly from data now available we may assign to six chromosomes the known single gene characters of rabbits. These are I, B-C; II, En-Du-L; III, A; IV, D; V, E; VI, V. Professor Painter informs me that the haploid number of chromosomes in the rabbit is 22. If we have identifying characters for only 6 of the 22, there remains a goodly number (16) to harbor genes of characters as yet undiscovered.

LINKAGE TESTS YIELDING POSITIVE RESULTS.

Linkage tests which have yielded positive results will be described first, those with negative results will be described later.

Linkage of English spotting with Angora (long) coat.

English spotting in rabbits (Plate 7) is a strongly dominant mutation which finds its expression in a complete inhibition of pigment formation in a considerable part of the coat. In a homozygous individual, the inhibition commonly extends to a larger part of the coat than in a heterozygous individual. The spots are placed for the most part symmetrically on the two sides of the body. They include typically, (1) a spot on either side of the nose, which may become confluent with a median nose spot; (2) a spot around the eye, frequently with a minor spot above and especially one below it; (3) a spot covering the entire ear, somewhat irregular and patchy near its base; (4) a lateral chain of round spots extending from the shoulder back along the side to the thigh; and (5) an interrupted median spinal stripe extending from the shoulders back to the tip of the tail. In heterozygotes the inhibition of pigment development is less complete than in homozygotes. In other words, the pigmented spots are larger and have a tendency to become confluent in certain regions. Thus the lateral nose spots fuse with each other, or with a median spot between them, producing the so-called "butterfly" marking. Also the eye-spots become larger and irregular in outline and bordering spots develop especially below and behind the eye-spot. A new spot frequently develops on the fore-leg in the radius-ulna region, and one on the hind-leg in the tibia-fibula region (Plate 7, fig. 2). The side chain may fuse into a nearly continuous lateral blanket, and spots appear on the belly marking the mammæ, and also a median ventral line.

By selection, as described in an earlier paper, I was able considerably to increase the extent of the spots, producing a very dark type of heterozygous English. (See Pl. 3, fig. 5, Castle, 1919.) This result, as I now believe, was probably achieved through the agency of modifying genes, although at the time I was more interested in establishing the fact of modification than attempting an explanation of it. The allelomorph of the English gene in my selected dark English race was the fully pigmented unspotted condition which is commonly known as self. For this allelomorph my selected animals were heterozygous, as are also ordinary exhibition English, since the more extensively spotted individuals are preferred to the less extensively spotted homozygous sort. Accordingly, when exhibition English rabbits are mated with each other, there is, as in the case of exhibition Dutch rabbits, a 50 per cent production of wasters. For 25 per cent of the young are true-breeding selfs, and 25 per cent are homozygous English (too white for the standard), while 50 per cent are heterozygous English, the type desired.

When English rabbits are crossed with Dutch, there are produced individuals which are heterozygous for both kinds of spotting. These are unmistakably English spotted but are considerably whiter than ordinary heterozygous English (Plate 7, fig. 3). They resemble homozygous English, particularly in the frequent absence of a nose spot and the reduction in extent of the other spots. Such rabbits have not been observed to produce self-colored young in F_2, hence it has been concluded that they do not contain the self allelomorph of English, but that Dutch has been substituted for it. On this view English, Dutch and self are a triple allelomorph system, a relation to which we shall need to give further attention.

One of the three positively established cases of genetic linkage in rabbits is between English spotting and Angora coat. Another is between Dutch spotting and Angora coat. These facts indicate that the genes for English, Dutch and Angora all lie in the same chromosome and make it seem likely that English and Dutch really are allelomorphs or closely linked with each other.

The evidence for the linkage of English and Angora is summarized in Table 9. It was obtained by the following procedure. English rabbits were crossed with self-Angoras. The English offspring, which were short-haired, were obviously heterozygous both for English and for Angora coat. They are mated with self-Angoras, the double recessive combination. Four classes of young were obtained, of which short-haired English and long-haired (Angora) selfs were greatly in excess, these being the original combinations, while the novel or cross-over combinations, long-haired English and short-haired selfs, were greatly in the minority. If there were no linkage, all four combinations should be equally frequent in occurrence. Since they were not, linkage evidently occurs. Its strength is indicated by the cross-over percentage, which is found by dividing the number of cross-over individuals by the total number of back-cross young.

Seven different F_1 males and ten F_1 females of the sort just described have been bred, some of them quite extensively, with double recessive mates, and the character of the young produced is indicated in Table 9, under the heading "repulsion." Male F_1 parents have produced 885 young, of which 59 were English long-haired and 66 self short-haired, these being the cross-over classes. Together they number 125, which is 14.12 per cent of the total, 885. Hence the cross-over percentage is 14.12 ± 1.13.

Table 9.—*Summary of back-cross tests for the existence of linkage between the genes for English spotting and angora coat (long hair).*

Doubly heterozygous parent.	Nature of cross.	Character of young.				Total.	Percentage cross-overs.	P. E.
		English short.	English long.	Self short.	Self long.			
♂ 4388	Repulsion	11	4	0	8	23	17.3	7.03
♂ 4595	Do	85	11	12	96	204	11.3	2.36
♂ 5002	Do	17	4	2	9	32	18.7	5.96
♂ 5053[a]	Do	12	3	4	19	38	18.4	5.47
♂ 5139[a]	Do	146	23	37	158	364	16.4	1.77
♂ 5165	Do	88	8	9	68	173	9.8	2.56
♂ 6299	Do	21	6	2	22	51	15.7	4.72
Totals:		380	59	66	380	885	14.12	1.13
♂ 5401	Coupling	6	13	16	1	36	19.4	5.62
♂ 5403	Do	2	10	15	3	30	16.6	6.16
Totals		8	23	31	4	66	18.18	4.15
♀ 4614	Repulsion	45	5	3	49	102	7.8	3.34
♀ 4615	Do	27	3	6	37	73	12.3	3.95
♀ 4596	Do	40	3	2	36	81	6.2	3.75
♀ 4597	Do	4	0	1	3	8	12.5	7.95
♀ 4960	Do	21	4	5	16	46	19.5	4.97
♀ 5004	Do	31	4	4	25	64	12.5	4.22
♀ 5054[a]	Do	11	3	4	26	44	16.1	5.08
♀ 5138	Do	26	7	3	25	61	16.4	4.32
♀ 5297[a]	Do	17	1	4	16	38	13.1	5.47
♀ 5348	Do	0	1	0	4	5	20.0	8.71
Totals:		222	31	32	237	522	12.06	1.48
♀ 5400	Coupling	5	30	15	3	53	18.6	4.63
♀ 5402	Do	1	11	19	3	34	11.7	5.78
Totals		6	41	34	6	87	13.79	3.62
Total, all series:						1560	13.07	0.85

The repulsion F_1 females have produced 522 young of which 31 long-haired English and 32 short-haired selfs constitute the cross-overs, these beings together 12.06 per cent of the total.

One of the long-haired English females, produced as a cross-over in the "repulsion" back-cross just described, was mated with a short-haired self rabbit, producing a litter of short-haired English young, so constituted that the English and Angora characters had been furnished by the *same* parent. Two males and two females (doubly heterozygous) of this litter were mated with double recessive animals (long-haired selfs) and their young show in every case *coupling* between English and Angora (see Table 9, Coupling). The males, 5401 and 5403, produced 66 young, of which 8 short-haired English and 4 long-haired selfs constitute the cross-over classes. They are 18.18 per cent of the total, 66, but of course in so small a total the probable error is large, namely 4.15 per cent. The females, 5400 and 5402, produced 87 back-cross young, of which the cross-over classes are 6 short-haired English and 6 long-haired selfs forming together 13.79 ± 3.62 per cent.

When the various back-cross matings from both the repulsion and the coupling series are combined, they show in a total of 1,560 young a cross-over percentage of 13.07 ± 0.85, which is equivalent to a linkage strength of 73.8 on a scale of 100.

In the last two columns of Table 9 is shown the cross-over percentage given by each individual parent, together with the probable error of that percentage. It will be observed that the deviation from the average, 13.07 per cent, is rarely greater than the probable error, and in no case equals twice the probable error. Hence it is reasonable to conclude that the deviations are due to chance alone and not to any peculiar genetic constitution of the parent. This is in harmony with the conclusion reached by Wachter from an extensive study of variations in the cross-over percentages among rats (see Castle and Wachter 1924).

The data contained in Table 9 afford an opportunity for comparison of the two sexes as regards frequency of crossing-over. In rats and mice all observations thus far recorded show crossing-over to occur a little more frequently in female than in male parents. But this is apparently not true in rabbits. The data of Table 9 show that male heterozygous parents have produced 951 young, with a cross-over percentage of 14.4 ± 1.09, whereas female heterozygous parents have produced 609 young with a cross-over percentage of 12.3 ± 1.37. Accordingly females have produced a *smaller* percentage of cross-overs (not a *larger*, as in rats and mice). The difference is 2.1 per cent ± 1.75. As it does not greatly exceed the probable error, it is of doubtful significance.

Linkage of Dutch spotting with Angora coat.

The Dutch type of spotting in rabbits is a recessive mutation (or series of mutations), in which the power of pigment production is not inhibited (as in English spotting) but nevertheless is apparently inadequate to develop pigment throughout the entire coat. In Plate 6 is shown a graded series of stages of Dutch markings in which the amount of white in the coat becomes progressively greater. All stages shown except the last, grade 18 (all white), have been observed. The all-white stage is probably not realized among Dutch rabbits, although it is found in homozygous Vienna-White rabbits, as a result of a genetically distinct mutation. In a previous publication

(Castle, 1919), I have shown that the fanciers' type of Dutch-marked rabbit, grade 8 of Plate 6, does not breed true, but is a heterozygote between two different types of Dutch, each of which may be obtained in a homozygous condition. These two types differ both in the amount of white in the coat and in its distribution. The type which I have called "dark" (Plate 5, fig. 2) has notably less white on the head and is characterized by a relatively narrow belt. In a homozygous state it fluctuates through grades 3 to 6. The other type, which I have called "white" (Plate 5, fig. 3), ordinarily fluctuates, when homozygous, through grades 10 to 17. There is reason to believe that certain genetic modifiers may lower or raise the range in both series, when introduced from an outcross. The White-Dutch type has two distinctive features. (1) The head pigmentation is relatively small in amount. It forms a separate patch on either side of the head, surrounding the eye and extending toward or on to the ear. The front of the iris is partially or wholly unpigmented (wall-eye). (2) The body pigmentation posterior to the white belt has a tendency to be broken up into fine spots. This feature is never present in a homozygous "dark" rabbit.

The heterozygote between dark and white types approximates grades 7 and 8, but it is unusual (1) for the two head spots to be of exactly equal size and even outline, (2) for the edge of the belt to be perfectly straight, and (3) for the hind feet to be white to exactly the same degree, all of which points the fanciers' standard insists upon. Therefore, while it is easy to produce the fanciers' type in quantity by crossing homozygous white with homozygous dark rabbits, the production of a prize-winner would probably remain, under this mode of procedure, as rare an event as it is under the common practice of breeding the heterozygous fanciers' type with its like.

In using Plate 6 as a grading scale, I have found the number of grades needlessly great in the region from 9 to 15, consequently there is a tendency in the records for counts to heap up on 12, 14 and 16, while the intermediate grades remain relatively low. There is in reality no discontinuity in the series, such as these apparent modes would suggest.

Soon after the appearance of my 1919 paper, in which the view was presented that the fanciers' type of Dutch rabbit is a heterozygote between two allelomorphic types of Dutch, a different interpretation was given to the case by Punnett (1920) and by Pap (1921), who have suggested explanations involving independent (not allelomorphic) factors. When the discovery was made that English is linked with Angora, it became evident that Dutch also, if it is allelomorphic with English or closely linked with it, should show linkage with Angora. To test this idea, I at once began crossing Dutch with Angora, using for the purpose such stocks of Dutch and Angora rabbits as I had on hand, and purchasing in addition a pair of standard-bred Dutch rabbits from a fancier who had recently won numerous prizes for the excellence of his stock. The newly acquired Dutch pair, to be known hereafter with their progeny as "Rice's" stock, were both grade 8 rabbits and proved, as expected, to be heterozygous for two types of Dutch, like those with which I had previously experimented. When mated with each other, they produced young as shown in the first entry of Table 10. The 7 young of grades 12 to 15 represent the homozygous White-Dutch individuals (WW) expected to be one-fourth of the total, actually 7 in 23. The expected 50 per cent of individuals heterozygous (DW), like their parents, are probably found chiefly among those of grades 7 to 9, actually numbering 10. The expected 25 per cent of homozygous dark (DD) individuals are probably found chiefly among those of grades 5 and 6, numbering 6. In harmony with this interpretation of the genetic constitution of the Rice's stock rabbits are the results of other matings of this same male. He was mated with certain female Dutch rabbits of my old stock, and of grades 2 and 3, known to be heterozygous for Self- and White-Dutch (♀ ♀ SW, Table 10) and produced two widely separated groups of young, (1) a group of 6 White-Dutch individuals of grades 13 to 15, and (2) a group of 10 individuals of grades 3 to 6. The former are certainly homozygous for the White type of Dutch. The latter should include three different gametic combinations, all equally numerous, namely DS, DW and SW. The expected grade of combinations DS and SW can be inferred from a third group of matings in which homozygous self-females (SS, Table 10) were used. These matings should produce only two types of zygotes, DS and SW, if the male is, as supposed, DW. The 75 young produced by such matings range in grade from 1 to 6, with modes on 2 and 4, the former being characteristic for DS and the latter for DW combinations, although the two undoubtedly intergrade.

Returning to the SW matings in Table 10, it is probable that the lower part of the range 3 to 6, shown by the three expected classes of heterozygotes, is occupied by the SD and SW combinations and that the DW combinations are found mostly in the higher grades, 5 and 6.

TABLE 10.—*Character (grade) of the young produced by the standard-bred Dutch male rabbit of Rice's stock in various matings.*

Mates.	Grade (see Plate 6.)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
♀ D W, grade 8, Rice's stock	..	..	..	..	2	4	4	5	1	..	..	1	1	3	2
♀ ♀ S W, grades 2 and 3	..	..	2	4	1	3	..	..	..	..	..	..	2	1	3
♀ ♀ S S, grade 0 (self)	5	22	19	22	5	2	..	..	..	..	..	..	..	..	..

The matings of Rice's male with SS females, recorded in Table 10, were made mostly with Angora self-females for the purpose of producing double heterozygotes with which to test for the existence of linkage between Dutch and Angora. Similar matings were made with Dutch rabbits of my older stocks remaining from the experiments described in 1919.

The first step toward the desired linkage tests was to mate *inter se* the double heterozygotes in the hope of obtaining double recessives, Angora-Dutch. Numerous matings of this sort were made, into the details of which we need not go, and several extracted White-Dutch were obtained, but none of these was Angora. This result suggested the existence of linkage, since the desired double recessive zygote, Angora-Dutch, would result only from the union of two cross-over gametes. If *one* cross-over gamete had entered into the production of any of the extracted White-Dutch, such an individual should be heterozygous for Angora. Accordingly, tests were made by mating the extracted White-Dutch individuals with Angoras. These resulted in showing that three of the F_2 White-Dutch (a female and two males) carried Angora as a recessive character, though they were themselves short-haired. Further, it was found that a grade 3 male Dutch Angora of the F_2 generation was heterozygous for White-Dutch, so that he also produced the desired type of gamete, White-Angora. By mating together or with F_1 individuals, the animals which were producing double recessive gametes, a stock of double recessive zygotes was at last obtained for use in back-crosses with F_1 individuals, the most satisfactory sort of linkage test.

The fact must be borne in mind that the Rice's stock male, whose matings are recorded in Table 10, was heterozygous for two types of Dutch, Dark and White, corresponding respectively with modal grades of 3 and 14. Consequently, when he was mated with self-Angoras, the F_1 individuals would some of them be heterozygous for Dark-Dutch and some of them for White-Dutch. In making back-cross linkage tests, the fact must be known *which* type of Dutch each F_1 animal carried. In some cases the F_2 litters indicated this, for any mating which produced White-Dutch individuals showed conclusively that both parents carried White-Dutch. F_1 individuals which had *not* produced White-Dutch when mated *inter se* could be tested more directly and conclusively by a cross with a White-Dutch (homozygous) individual. By tests of one sort or the other all F_1 individuals were finally listed as carrying either Dark- or White-Dutch as the allelomorph of self, the somatic expression of this combination being in grade from 1 to 6, usually 2 to 4, as shown in Table 10. We shall deal with the two sorts of heterozygotes separately, and first shall deal with White-Dutch, since its various kinds of combinations are easier to distinguish.

Because of the scarcity at first of double recessive individuals, the tests were usually made between double recessive males and F_1 females, since the same male could be used with a large number of females. The first double recessive male which became available for these tests was ♂ 5590, a grade 14 Angora rabbit, homozygous, it will be remembered, both for the White type of Dutch and for Angora coat. He was mated with F_1 females produced by mating short-haired Dutch with long-haired self rabbits, in which, accordingly, Dutch and Angora would be expected to repel each other, if they are linked. The female mates of ♂ 5590 were of various grades ranging from 0 to 5, although all of them were known to be carriers of White-Dutch, and their progeny are tabulated separately according to the grade of the mother, in Table 11. It will be observed first that about half of the young are long-haired and half of them short-haired, as expected.

There were actually 86 Angoras and 81 short-haired young. But most of the Angoras are of low grade (1 to 5) like their heterozygous mothers, while nearly all the short-haired young are of higher grade, resembling their homozygous father. Actually, only 9 of the 86 Angora young are whiter than grade 5, whereas all except 13 of the 81 short-haired young are whiter than grade 5. The average grade of the Angoras is 3.4, that of the short-haired young is 9.2.

This result shows unmistakable linkage between White-Dutch and Angora coat. The two characters evidently repel each other in the gametogenesis of the heterozygous females. It is impossible to say just how many of the young recorded in Table 10 are genetically homozygous White-Dutch, and how many are genetically heterozygous for White-Dutch and self, since the two classes evidently intergrade (phenotypically). If we assume provisionally that all animals as dark as the mothers (known to be heterozygous) are like them heterozygous, and that all which are whiter than the mothers are extracted homozygous White-Dutch, the 167 young of Table 10 will fall into four groups as follows:

Table 11.—*Classification of the back-cross young produced in crosses between F_1 doubly heterozygous females and the double recessive Dutch-Angora male, 5590, grade 14.*

Grade of F_1 mother.	Grade of Angora young.														Total Angora.	Av. grade.
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
0......	2	2	..	...	1	...	...	...	...	...	...	...	...	...	5	2.5
1......	10	7	5	...	...	...	...	...	1	...	...	...	...	...	23	2.1
2......	...	1	4	...	1	...	...	...	...	...	...	...	...	...	6	3.1
3......	2	11	7	3	3	1	1	...	...	1	...	...	...	1	30	3.6
4......	...	2	2	2	1	1	...	...	...	...	...	1	...	...	9	4.5
5......	...	3	3	...	5	...	...	...	...	...	...	1	1	...	13	5.0
Totals:	14	26	21	5	11	2	1		1	1		2	1	1	86	3.4

Grade of F_1 mother.	Grade of short-haired young.																Total short.	Av. grade.
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
0......	...	...	...	...	...	...	...	2	...	...	...	...	...	...	...	...	2	8.0
1......	1	2	...	...	...	1	5	4	3	5	3	4	1	...	...	...	29	8.6
2......	...	...	...	1	...	...	1	...	1	4	...	2	...	1	...	...	10	9.8
3......	1	1	...	...	3	1	...	3	1	2	1	7	1	2	...	...	23	9.2
4......	...	...	2	1	1	...	...	...	...	2	1	3	...	...	...	...	10	8.2
5......	...	...	...	...	...	...	1	...	...	...	...	1	...	4	...	1	7	13.0
Totals:	2	3	2	2	4	2	7	9	5	13	5	17	2	7	...	1	81	9.2

SW Angora	WW Angora	SW Short	WW Short
77	9	13	68

The two middle groups are obviously cross-overs, comprising 22 of the 167 young, or 13.17 per cent of the total. No great importance is for the present attached to these figures, since it is possible that the limits of the groups are not correctly placed, but the indication of linkage is strong.

The next double recessive male to become available was 5941, also a grade 14 Dutch-Angora (Table 12). He was mated with a large number of doubly heterozygous females of the "repulsion" sort, produced by crossing self Angoras with Dutch short-haired rabbits, and themselves ranging in grade from 0 (self) to 6. These females produced 568 young of which 268 were Angora and 300 short-haired. A detailed classification of the young is given in Table 12. It will be observed that the somatic character of the mother influences that of her young, as was clearly shown in my 1919 paper. The average *grade* of the young rises with that of the mother, the father being in every case the same. In Tables 11 and 13 also, the same point is illustrated. But every mother, whatever her somatic grade, produces for the most part low grade (heterozygous for Dutch) Angoras, and high grade (homozygous for Dutch) short-haired young. The average grade of the former is 3.2, that of the latter being 10.6. The unmistakable cross-overs are the whiter Dutch-Angoras (grades 8 to 16) and the darker Dutch short-haired individuals (grades 0 to 5). It is impossible, without individual breeding tests, to assign the young in the region of possible intergrading with certainty to their proper genetic classes. We might, in our statistical treatment, either omit them altogether, or divide them between the intergrading groups. Either procedure would give about the same cross-over percentage, the thing we desire to estimate as accurately as possible. I think we shall not go far astray if we place in the group of heterozygotes (SW) all young of grade 6 or lower, since the mothers known to be heterozygous have this same range, and in the group of homozygotes (WW) all of grade 7 or higher. On this basis the groups become as follows:

SW Angora	WW Angora	SW Short	WW Short
244	24	44	256

Theoretically the two cross-over classes should be equal, as should also the two non-cross-over classes. But there is frequently a small excess of short-haired over Angora young, owing probably to a greater survival value. Accordingly, it would be impossible to place the limits of the groups so as to equalize these classes. If we placed the dividing point between grades 5 and 6, it would make a difference of only one in the total of cross-overs in Table 12. The groups would then be 240 : 28 : 39 : 261, a fairly symmetrical distribution. Either of the groupings suggested would give an indicated cross-over percentage close to 12, the former giving 11.9 per cent, the latter 11.8 per cent. The probable error in the cross-over percentage for 568 young is 1.41 per cent.

The third double recessive Angora-Dutch male used in the back-cross experiments was 6248 (Table 13), a son of 5941 of Table 12. He was of grade 16, his sire being of grade 14. He was mated with doubly heterozygous females of grades 1 to 6, many of them the same individuals as were mated with males 5590 and 5941 (Tables 11 and 12). The young are classified in Table 13. It will be observed that 81 of the young were Angora and 89 short-haired. The average grade of the young is higher in this case than

TABLE 12.—*Classification of the back-cross young of the double recessive Dutch-Angora male, 5941, grade 14.*

Grade of F_1 mother.	Grade of Angora young.																	Total Angora.	Av. grade.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
0.........	1	2	3	2	..	1	..	..	1	..	..	..	..	..	..	..	..	10	2.7
1.........	4	40	14	8	5	1	2	1	1	..	..	..	2	1	..	..	..	79	2.3
2.........	1	9	14	17	4	1	..	1	..	..	2	..	..	..	3	..	..	52	3.4
3.........	..	10	14	18	3	5	..	..	..	..	2	2	1	..	1	..	..	56	3.5
4.........	..	4	10	13	3	5	1	..	..	..	..	..	1	..	..	1	..	38	3.5
6.........	..	5	3	7	10	3	1	1	..	1	..	..	1	..	..	..	1	33	4.1
Totals:	6	70	58	65	25	16	4	3	2	1	4	2	5	1	4	1	1	268	3.2

Grade of F_1 mother.	Grade of short-haired young.																		Total short.	Av, grade.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
0.........	1	4	1	1	..	1	2	..	3	1	6	1	5	..	..	..	..	..	26	7.3
1.........	..	..	4	2	1	4	3	5	6	3	11	6	19	1	8	2	3	1	79	10.0
2.........	..	1	1	1	..	1	..	3	5	1	3	4	15	2	8	3	2	..	50	11.0
3.........	..	1	3	1	2	3	..	2	3	3	8	1	11	3	13	3	2	..	59	10.5
4.........	1	1	2	1	..	1	..	..	2	1	..	4	10	1	14	4	2	..	44	11.4
6.........	..	..	..	..	..	..	..	..	1	4	5	2	11	2	8	2	7	..	42	12.5
Totals:	2	7	11	5	3	10	5	10	20	13	33	18	71	9	51	14	16	1	300	10.6

in Table 12, corresponding with the higher grade of the sire. The Angora young average 5.6 in grade, those of Table 12 averaging 3.2. The short-haired young average 12.2, those of Table 12 averaging 10.6. It seems justifiable, accordingly, to advance by about two grades the dividing point between the cross-over and non-cross-over classes, including among the heterozygotes (SW individuals) all of grade 8 or lower, and among the homozygotes all of grade 9 or over. This procedure makes the groups as follows:

SW Angora	WW Angora	SW Short	WW Short
69	12	19	70

Placing the dividing point one grade lower or one grade higher would add one to the estimated cross-overs. Placing it two grades lower, between 6 and 7, as in Table 12, would add 3 to the estimated cross-overs and make the groups 60 : 21 : 13 : 76, a less symmetrical distribution. The one first suggested is therefore probably the best. It makes the indicated cross-over percentage 18.2. Any other grouping would increase the percentage.

The fourth double recessive Dutch-Angora male used in back-cross tests was 6330, grade 17 (Table 14), a son of 6248 (Table 13). He has been mated with some of the same (repulsion) females whose young are classified in Tables 10 to 12. The female mates were of various grades (1 to 6) and in this series of matings have produced 53 young classified in Table 14. The cross-over and non-cross-over classes are here easily recognized and do not intergrade. They are as follows:

SW Angora	WW Angora	SW Short	WW Short
20	4	6	23

This is a symmetrical distribution similar to those of Tables 11 to 13, and with the usual slight excess of short-haired over Angora individuals. The indicated cross-over percentage is 18.1 ± 4.5.

If now we combine the results obtained from the four double recessive males (Tables 11 to 14), we have the following groupings of the young:

SW Angora	WW Angora	SW short	WW short
410	49	82	417

The two middle (cross-over) classes together number 131 in a total population of 958, indicating a cross-over percentage of 13.6 ± 1.09. This, it should be borne in mind, is the crossing-over in oögenesis, that is the result given by doubly heterozygous females in back-crosses with double recessive males, the characters having entered the cross from different parents and showing repulsion in gametogenesis.

A small series of matings has been made between the same double recessive males and doubly heterozygous females so constituted as to show coupling. In this case the females resulted from a cross between a Dutch-Angora individual and a self-short-haired one. The young may be grouped in the same way as the young of females showing repulsion (Tables 11–14, where the same males were used). The groups are as follows:

TABLE 13.—*Classification of the back-cross young of the double recessive, Dutch-Angora male, 6248, grade 16*

Grade of F_1 mother.	Grade of Angora young.																	Total Angora.	Av. grade.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
1.........	3	2	2	9	8	1	1	1	1	1	..	..	2	..	..	1	1	33	4.7
2.........	..	..	..	2	6	2	3	1	2	..	..	..	..	1	2	1	..	20	6.8
3.........	..	1	..	..	2	..	1	1	..	..	..	..	..	..	..	..	..	5	4.5
4.........	..	..	..	1	2	2	1	1	1	..	..	..	..	..	..	..	..	8	5.2
5.........	..	..	..	2	1	4	1	..	..	..	..	..	..	..	1	..	1	10	6.6
6.........	..	..	..	1	1	..	1	..	1	..	..	..	1	..	..	..	..	5	6.6
Totals:	3	3	2	15	20	9	8	4	5	1	..	..	3	1	3	2	2	81	5.6

Grade of F_1 mother.	Grade of short-haired young.																		Total short.	Av. grade.
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
1.........	..	..	..	..	..	..	1	..	1	2	2	..	12	..	4	2	3	1	28	12.4
2.........	..	1	..	1	..	..	..	..	..	..	..	..	2	..	1	2	3	1	11	12.4
3.........	..	..	..	..	..	1	..	..	3	..	..	..	..	1	1	3	1	..	10	11.7
4.........	..	..	..	2	..	..	2	..	..	..	..	..	3	..	5	1	5	..	18	12.1
5.........	..	..	..	..	..	..	..	2	..	..	..	..	..	..	3	..	5	1	11	13.9
6.........	..	..	..	1	1	2	1	..	..	..	..	..	..	..	1	2	1	2	11	10.6
Totals:	..	1	..	4	1	3	4	2	4	2	2	..	17	1	15	10	18	5	89	12.2

TABLE 14.—*Classification of the back-cross young of the double recessive Dutch-Angora male, 6330, grade 17.*

Grade of F_1 mother.	Grade of Angora young.															Total Angora.	Av. grade.
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
1–6.......	2	10	3	4	1	..	..	..	..	1	1	..	..	1	1	24	5.2

Grade of F_1 mother.	Grade of short-haired young.																	Total short.	Av. grade.
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
1–6.......	1	..	..	4	1	..	..	..	..	1	7	4	..	4	1	4	2	29	11.2

Sire.	SW Angora.	WW Angora.	SW short.	WW short.
♂ 5941	1	6	7	4
♂ 6248	2	14	20	2
♂ 6330	1	3	16	3
Totals	4	23	43	9

It will be noticed that the groups which were largest in the repulsion crosses are here smallest, and *vice versa,* for the classes, which in the repulsion series were cross-overs, are here non-cross-overs and *vice versa.* There are 13 cross-overs in the total of 79 young which makes the percentage of cross-overs 16.4 ± 3.8, in fair agreement with the results for the repulsion series, which averaged 13.4 ± 1.1 per cent cross-overs.

So far we have dealt only with crossing-over in heterozygous females. Some observations have also been made on crossing-over in F_1 males doubly heterozygous for Dutch and Angora, in which the characters show coupling. Mated with double recessive females of grades 9 to 16, three F_1 self-males (grade 0) have produced 123 young, as shown in Table 15.

TABLE 15.—*Classification of the back-cross young produced by double recessive Angora-Dutch females of grades 9–16, when mated with F_1 doubly heterozygous males of grade 0.*

<table>
<tr><th colspan="16">Grade of Angora Young.</th><th rowspan="2">Total Angora.</th><th rowspan="2">Av. grade.</th></tr>
<tr><td>0</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>8</td><td>9</td><td>10</td><td>11</td><td>12</td><td>13</td><td>14</td><td>15</td></tr>
<tr><td>1</td><td>4</td><td>...</td><td>1</td><td>1</td><td>2</td><td>3</td><td>5</td><td>1</td><td>3</td><td>14</td><td>5</td><td>8</td><td>2</td><td>7</td><td>1</td><td>58</td><td>9.3</td></tr>
<tr><th colspan="16">Grade of short-haired young.</th><th rowspan="2">Total short.</th><th rowspan="2">Av. grade.</th></tr>
<tr><td>0</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>8</td><td>9</td><td>10</td><td>11</td><td>12</td><td>13</td><td>14</td><td></td></tr>
<tr><td>10</td><td>30</td><td>11</td><td>2</td><td>...</td><td>1</td><td>...</td><td>1</td><td>1</td><td>...</td><td>3</td><td>2</td><td>3</td><td>...</td><td>1</td><td></td><td>65</td><td>2.7</td></tr>
</table>

Classifying back-cross young of grade 5 or lower as heterozygous Dutch and those of Grade 6 or higher as homozygous Dutch (a procedure justified by the low grade and breeding performance of the F_1 males) we obtain groups as follows:

SW Angora	WW Angora	SW Short	WW short
9	49	54	11

The cross-over classes are the SW Angora and WW short young. Together they number 20 in a total of 123 young, which is 16.2 ± 3.04 per cent cross-overs, a result in good agreement with that given by F_1 females in the same kind of mating.

Another F_1 male, 5300, grade 4, of the repulsion sort, was mated with double recessive, Angora-Dutch females of grades 12 to 14, and produced 53 young grouped as follows, on the supposition that young of grade 6 or lower are heterozygous Dutch, those of grade 11 or higher being homozygous (none occurring of intermediate grades):

SW Angora	WW Angora	SW short	WW short
28	3	6	16

The two middle groups here represent cross-overs and the indicated cross-over percentage is 16.98 ± 4.63, a result in good agreement with that given by other F_1 heterozygotes in similar tests.

Combining now all the data on linkage between White-Dutch and Angora obtained from back-crosses of doubly heterozygous individuals, whether male or female, we have a grand total of 1,213 young, of which 173 may be regarded as cross-overs, which makes the estimated percentage of cross-over gametes 14.26 ± 0.96.

As regards the question whether crossing-over occurs more frequently in one sex than in the other, the data from the Angora-Dutch linkage tests point in the same direction as the data from the Angora-English test, but like them are inconclusive. (See Table 16.) They suggest that, in the rabbit, crossing-over occurs a little more frequently in males than in females, which is just the opposite of what has been shown to be true for rats and mice.

F_1 males have produced 176 back-cross young, 29 of which were cross-overs, a percentage of 16.4 ± 2.54. F_1 females have produced 1,037 back-cross young of which 144 were cross-overs, a percentage of 13.8 ± 1.05. The difference is 2.6 ± 2.7, which, like the difference found in the Angora-English tests, is devoid of statistical significance, since it scarcely exceeds the probable error.

A comparison of the linkage strength between Angora-Dutch and Angora-English (Table 16) would seem to indicate that the gene for English lies between the other two genes, distant one or two units from Dutch. On this basis, the linkage-map for males would have to be made *longer* in all its parts than for females, whereas in rats and mice the cross-over values for females are consistently higher than for males. The matter merits further investigation, but the evidence already in hand would not lead us to expect to find any uniform sex-difference in linkage throughout the class of mammals. Moreover, there is no statistically significant evidence that English and Dutch are not at one and the same genetic locus, since the differences in their linkage values with Angora exceed by very little the probable error for the number of observations made.

TABLE 16.—*Comparative cross-over values for Angora-Dutch and Angora-English linkages, in the two sexes.*

	F_1 males.	F_1 females.	Difference.
Angora-Dutch	16.4 ± 2.54	13.8 ± 1.05	2.6 ± 2.75
Angora-English	14.4 ± 1.09	12.3 ± 1.37	2.1 ± 1.75
Difference	2.0 ± 2.76	1.5 ± 1.72	

Evidence has been obtained, since the foregoing was written, to show that English and Dutch *are* at different genetic loci, which, however, are very close together.

Linkage between Dark-Dutch and Angora.

Thus far in our discussion of the linkage between Angora and Dutch, we have been dealing wholly with White-Dutch. I have stated that in crossing Rice's stock of Dutch rabbits with self-Angoras, two sorts of heterozygotes were obtained, one sort carrying White-Dutch (Plate 5, fig. 3) as the allelomorph of self, the other carrying Dark-Dutch (Plate 5, fig. 2). By suitable tests (mating to homozygous White-Dutch) the two sorts of heterozygotes can be distinguished, even when their somatic character is not conclusive. Such tests were made and the genetic constitution of each F_1 animal duly ascertained. What was done with the heterozygotes that were found to carry White-Dutch has already been described. It remains to describe the experiments which were made with those carrying Dark-Dutch.

A grade 2 Dutch male, which had received Dark-Dutch from his sire, the original Rice's stock male, and self from his Angora mother, was mated with nine Angora females carrying Dark-Dutch. Seven of the females were of grade 3, one of grade 4, and one of grade 1. As the young of all mothers were very much alike in grade, they will be included in one summary. (See Table 17, ♂ 5585.) These matings produced 22 Angora and 19 short-haired young. The Angoras ranged in grade from 0 to 5, the shorts from 1 to 6. The average grade of the former was 2.3, of the latter 3.0. Neither group has a clearly bimodal distribution or a range sufficiently extended to permit distinguishing cross-over from non-cross-over classes. If such classes occur, they evidently intergrade. What evidence, if any, have we that this type of Dutch is linked with Angora? Only the average grade of the two classes of young, but this is highly significant.

Consider for a moment the genetic constitution of each parent. The father is known by pedigree and breeding tests to have received from his sire Dark-Dutch and short coat, and to have received from his mother self and Angora. He should produce gametes, half of which transmit Dark-Dutch and half of which transmit self. If there is linkage, short hair should go oftener with Dutch than with self to his progeny. His mates are Dark-Dutch Angoras. They will transmit Angora in every gamete.

It is immaterial for the experiment whether the mothers transmit Dutch in all their gametes, or in only half of them, since each sort will be equally likely to go to short-haired or to Angora young, all depending on the combination transmitted by the doubly heterozygous sire. If, then, the short-haired young produced by this cross are of higher grade than the Angora young, it can only be because the sire transmits Dutch *oftener associated with short hair than with Angora,* in other words it will be evidence of linkage.

Now, when the young were graded (at birth), it was unknown which ones would be short-haired and which Angoras, since the two sorts can not

TABLE 17.—*Classification of the back-cross young produced in matings designed as tests for linkage between Dark-Dutch and Angora.*

Nature of mating.	Grade of Angora young.										Total Angora.	Av. grade.
	0	1	2	3	4	5	6	7	8	9		
S D ♂ 5585, grade 2, × D D Angora ♀ ♀, grade 1–4	1	6	7	2	5	1	..	..	..	..	22	2.31
W W ♂ 5990, grade 14, Angora, × ♀ ♀ S D, grade 0–4	1	7	11	10	3	1	..	..	..	..	33	2.30
W W ♂ 5941, grade 14, Angora × ♀ ♀ S D, grade 0–6	7	11	25	18	14	5	..	..	..	1	81	2.53
W W ♂ 6248, grade 16, Angora, × ♀ ♀ S D, grade 2–6	..	2	2	5	2	5	1	1	..	1	19	4.00
Totals:	9	26	45	35	24	12	1	1	..	2	155	2.63

Nature of mating.	Grade of short-haired young.											Total short.	Av. grade.
	0	1	2	3	4	5	6	7	8	9	10		
S D ♂ 5585, grade 2, × D D Angora ♀ ♀, grade 1–4	..	3	6	4	4	1	1	..	..	..	..	19	3.00
W W ♂ 5990, grade 14, Angora, × ♀ ♀ S D, grade 0–4	..	1	3	5	3	4	..	..	..	..	..	16	3.37
W W ♂ 5941, grade 14, Angora × ♀ ♀ S D, grade 0–6	3	6	7	21	8	6	4	2	..	..	..	57	3.21
W W ♂ 6248, grade 16, Angora, × ♀ ♀ S D, grade 2–6	..	..	1	5	4	2	..	1	..	2	1	16	4.87
Totals:	3	10	17	35	19	13	5	3	..	2	1	108	3.41

be distinguished until they are about 3 weeks old. This fact I mention to show that the result is unbiased by any hypothesis held by the observer when the grading was done. It will be noted that the short-haired young produced by this male are really of slightly higher average grade than the Angora young, an indication of linkage.

The same result is given in three other experiments (Table 17) in which doubly heterozygous females were mated with Angora males. The females were either sisters of ♂ 5585, or produced by similar matings. They were doubly heterozygous for Angora and for Dark-Dutch. They were mated with males homozygous for White-Dutch as well as for Angora, the same males in fact which were used as the double recessive parents in Tables 11 to 13.

The mothers in these crosses would form only two kinds of gametes, if no cross-overs occurred, viz, *self-Angora* and *Dark-Dutch* (grade 1 to 6) *with short-hair.* When crossing-over occurred, self might become associated with short-hair, and Dark-Dutch with Angora, but these combinations would be in a minority, if Dark-Dutch is linked with Angora. Now it is impossible to distinguish sharply the two kinds of heterozygotes, (1) Dark-Dutch-White-Dutch and (2) Self-White-Dutch. In Rice's stock, the former varies around grade 8, the latter (in F_1 cross-breds) from 1 to 6. But in the presence of other genetic factors (modifiers ?) the SW heterozygote may be a self animal (grade 0), and the DW combination may be considerably lowered in grade from the modal condition, 8, of fancy rabbits. Nevertheless, other things being equal, that is in the same stock or with the same set of genetic modifiers present, SW is of *lower* grade than DW. (See also my 1919 paper.) This being so, the Angora young in these back-crosses should be of lower grade than the short-haired young, if linkage occurs between Dark-Dutch and Angora, since SW zygotes would prevail among the Angoras and DW zygotes among the short-haired. That such is the case is shown by the fact that in every experiment (Table 17) the Angora young are of lower grade than the short-haired young. The average grade of the 155 Angora young is 2.63, that of the 108 short-haired young is 3.41.

Our conclusion from these experiments is that Dark-Dutch (as well as White-Dutch) is certainly linked with Angora. We can not calculate any cross-over percentage, because we can not, without extremely tedious breeding tests, determine how many of the back-cross young have resulted from cross-overs. But this is not necessary. In my 1919 paper, evidence was presented indicating that the two types of Dutch, "White" and "Dark," commonly found together in fancy Dutch rabbits, are allelomorphs. The fact that they both show linkage with the same third character (Angora coat) supports that interpretation, since it shows that their genes lie in the same chromosome. It does not, of course, show that their genes occupy the same genetic locus, but it does effectually disprove the hypothesis of independent major factors for Dutch.

Relation of English to Dutch.

It has been shown that both English and Dutch are linked with Angora, and that the linkage strengths are similar in both cases. This indicates either that the genes for English and Dutch are close to the same locus, or that they are on opposite sides of Angora and about equally distant from it. The first-mentioned alternative is strongly supported by observations reported in 1919, in which Dutch and English were shown to behave like allelomorphs, since no cross-overs were detected in a total of 193 back-cross young.

A renewed investigation of the matter has since been made, the Angora character being introduced into the cross between English and Dutch, as an aid in detecting cross-overs. Short-haired English rabbits have been crossed with long-haired Dutch, resulting in the production of short-haired English progeny with colored areas less extensive than those of English rabbits free from Dutch as a recessive character (Plate 7, fig. 3). These F_1 animals, heterozygous for three characters, English, Dutch, and Angora, have been back-crossed with triple recessives, ordinary Dutch-Angoras (which are non-English). There have been produced 537 back-cross young, as shown in Table 17A, of which 274 are clearly Dutch and 263 clearly English. One of the latter is also unmistakably homozygous for Dutch. This is indicated (1) by the greatly reduced colored areas of the coat, which are less than those of either an ordinary homozygous English or an ordinary homozygous Dutch. The entire coat is white except a small spot round each eye and a part of each ear. The pigmentation at the base of the tail, which is invariably present in even the whitest ordinary Dutch, is in this animal entirely wanting. (2) A second evidence that this individual is homozygous for White-Dutch is the fact that its eyes are "wall," a character always found in homozygous White-Dutch, but never hitherto observed in an English rabbit.

This evidence is supported by breeding tests of the animal in question, which fortunately is a male. Mated with homozygous White-Dutch females he has produced four litters of young, part of which are ordinary White-Dutch and others very light English like himself, with no body spots whatever and very restricted head spots (eye and ear).

Since the animal has an Angora coat, it is evident that he is homozygous for this recessive character also. Accordingly, it is clear that his F_1 father contributed to his production, a gamete bearing English, Dutch, and Angora. This can have arisen only (1) by a cross-over between English and Dutch, which has brought the genes of both into a single chromosome, or else (2) by a non-disjunction between the chromosomes bearing these genes, which would make this animal triploid for that particular chromosome. Further experiments are in progress to test these alternative explanations.

If, as seems probable, English and Dutch are not allelomorphs but have genes at closely adjacent loci, the question arises, what is the order of the

Table 17A.

F_1 parent.	Short Dutch.	Angora Dutch.	Short English.	Angora English.	Angora Dutch English.	Total.	Percentage cross-overs between Dutch and Angora.	Percentage cross-overs between English and Angora.
♂ 26	5	22	27	5	0	59	18.5	15.6
♂ 60	2	22	21	6	0	51	8.3	22.2
♂ 201	25	182	165	18	1	391	12.0	9.8
Total:	32	226	213	29	1	501	12.3	12.2
♀ 200	0	7	15	0	0	22	0	0
♀ 307	1	1	2	0	0	4	50.0	0
♀ 322	1	6	3	0	0	10	14.3	0
Total:	2	14	20	0	0	36	12.5	0
Both sexes	34	240	233	29	1	537	12.3	11.4

genes. Does English lie between Dutch and Angora or does Dutch occupy the middle position? The possibility that Angora occupies the middle position is excluded by the close linkage of English and Dutch with each other and the relatively loose linkage of each with Angora. Linkage tests En-L and L-Du, as summarized in Table 21, indicate that English is nearer to Angora than Dutch is, since the cross-over percentage in the former case is 13.0 ± 0.8 and in the latter 14.2 ± 0.9. The data of Table 17A indicate the same relation, though the difference between the two cross-over values is slightly less. This evidence is particularly convincing because it is based on matings in which both relations are simultaneously being tested.

But if the order of the genes is as indicated Du-En-L, then the cross-over individual already described must have arisen by a *double* crossing-over, since the two end genes of the series came from one parent and the middle gene from the other, in making up the new combination which he represents. On the other hand, if the new combination of characters in one gamete arose through non-disjunction of this chromosome pair (in the F_1 parent), one of which bore English and short-hair, the other Dutch and Angora, then it would not be necessary to suppose any crossing-over to have occurred and English and Dutch might be at identical loci. The former interpretation seems to me the more probable but the latter is not as yet excluded.

The "map distance" between En and Du is indicated by the respective distances En-L and Du-L in Table 21 to be 1.2 ± 1.2, by the corresponding data in Table 17A to be 0.9 ± 1.46. But the single apparent cross-over between English and Dutch is recorded as a unique event in the production of 730 back-cross young, in which this particular event was being looked for. This is a frequency of 0.1 per cent, which indicates a very close linkage between the genes in question.

Linkage of Brown Pigmentation with Albinism and its Allelomorphs.

In studying the linkage relations of albinism and its allelomorphs, in particular of chinchilla, with other factors, only one positive association has been discovered and that pertains to brown, the recessive allelomorph of black pigmentation.

My original chinchilla rabbits were black pigmented. When they were crossed with brown Himalayans, black chinchilla young were obtained showing the two dominant characters. These double heterozygotes, when back-crossed with the double recessive variety, produced the four expected classes of young in unequal proportions, the cross-over classes, brown chinchilla and black Himalayan, being regularly smaller than the original combinations or non-cross-over classes, black chinchilla and brown Himalayan. (See Table 18.)

TABLE 18.—*Classification of the back-cross young produced by mating F_1 rabbits heterozygous for brown and for chinchilla, with double recessives (brown Himalayan).*

F_1 parent.	Black chinchilla.	Brown chinchilla.	Black Himalayan.	Brown Himalayan.	Total.	Cross-over percentage.
♀ 29	5	4	3	4	16	22.8
♀ 57	3	3	3	3	12	50.0
♀ 135	3	1	1	3	8	40.0
♀ 158	19	15	13	21	68	41.4
♀ 224	6	2	2	0	10	40.0
♀ 225	3	3	1	7	14	28.5
♀ 264	22	12	15	29	78	34.6
♀ 266	12	4	3	8	27	25.9
♀ 269	11	14	4	11	40	45.0
♀ 324	18	12	12	15	57	42.1
♀ 326	31	13	9	23	76	28.9
♀ 332	18	11	9	25	63	31.7
♀ 419	8	3	2	4	17	29.4
♀ 439	15	8	3	6	32	34.3
Totals	174	105	80	159	518	35.7
♂ 82	9	1	1	6	17	11.8
♂ 101	13	7	3	14	37	27.0
♂ 261	6	5	5	5	21	47.6
♂ 274	42	16	20	49	127	33.6
Totals:	70	29	29	74	202	28.7
Both sexes:	244	134	109	233	720	33.75

Fourteen F_1 females have produced 518 back-cross young, of which 185, or 35.7 per cent, were cross-overs. Also 4 F_1 males have produced 202 back-cross young of which 58, or 28.7 per cent, were cross-overs. Combining the results for both sexes, the F_1 parents have produced 720 back-cross young, of which 243, or 33.75 ± 1.26 per cent, were cross-overs. All except one of the 18 F_1 parents tested have produced less than 50 per cent of cross-overs, thus giving indications of linkage. The single exception is ♀ 57 which has produced only 2 litters, 12 young, of which exactly half are cross-overs. There can accordingly be no reasonable doubt that linkage exists between chinchilla and brown.

Since evidence has been produced that chinchilla is an allelomorph of full color as well as of albinism, it is to be expected that linkage will be found to exist between the other allelomorphs of chinchilla and brown. To test this, a cross was made between a brown chinchilla, produced as a cross-over in the experiment just described, and a fully colored black individual. In this experiment it was to be expected that chinchilla and brown would show coupling, that is, would preferentially *go together*, instead of repelling each other as in the previous experiment. This expectation was realized as shown in Table 19.

Table 19.—*Classification of the back-cross young produced by mating F_1 rabbits heterozygous for brown vs. black and for chinchilla vs. full color, with homozygous brown Himalayans.*

F_1 parent.	Black full color.	Brown full color.	Black chinchilla.	Brown chinchilla.	Total.	Percentage cross-overs.
♀ 70	10	2	6	7	25	32.0
♀ 122	4	7	2	9	22	40.9
♀ 138	7	3	6	6	22	40.9
♀ 253	5	4	2	3	14	42.8
♀ 438	6	8	3	9	26	42.3
Total:	32	24	19	34	109	39.4 ± 3.2
♂ 63	20	7	10	16	53	32.0 ± 4.6
Both sexes:	52	31	29	50	162	37.0 ± 2.6

Coupling between brown and chinchilla was found to exist among the back-cross young of each of the 6 F_1 parents tested, of which 5 were females. The cross-over percentage for the 162 young raised from this cross was 37.0 ± 2.6.

A cross reciprocal to that just described was made between a full-colored brown (chocolate) individual and a black-pigmented chinchilla. An F_1 female from this cross, an ordinary gray in appearance, was mated with a brown Himalayan male, producing 69 young as follows:

Black full-color.	Brown full-color.	Black chinchilla.	Brown chinchilla.
11	22	21	15

It will be observed that the cross-over classes in this experiment (black full-color and brown chinchilla) are those which were non-cross-overs in the reciprocal experiment (Table 19). The cross-over percentage is 37.6 ± 4.0, in good agreement with the result of the reciprocal experiment.

Finally, a cross was made which did not involve chinchilla, but two of its allelomorphs, namely full-color and Himalayan albinism. The full-colored parent was black and the Himalayan parent brown. The young were of course full-colored and black-pigmented. Three F_1 females were back-crossed with the double recessive variety, brown Himalayan, producing young as follows:

F_1 parent.	Black full-color.	Brown full-color.	Black Himalayan.	Brown Himalayan.	Total.
♀ 150......	5	3	2	9	19
♀ 273......	3	1	5	1	10
♀ 352......	8	2	4	6	20
Total:	16	6	11	16	49

The cross-overs number 17, or 34.7 ± 4.8 per cent, a linkage strength comparable with that found in other experiments of the series. This result confirms the evidence from other sources that chinchilla is an allelomorph of full-color as well as of albinism.

If we combine all the experiments designed to test the strength of linkage between chinchilla (or one of its several allelomorphs) and brown, we have a total of exactly 1,000 back-cross young of which 346, or 34.6 ± 1.07 per cent are cross-overs. Considering the sexes separately, F_1 females have produced 745 young, of which 271, or 35.9 ± 1.2 per cent, are cross-overs. F_1 males have produced 255 young, of which 75, or 29.8 ± 2.1 per cent are cross-overs. Females here give a higher percentage of cross-overs, as is the case among rats and mice. The difference between the sexes is 6.1 per cent. Its probable error is 2.4 per cent, which is 2.5 times the probable error, a difference of doubtful significance, and this view of the matter is strengthened by the consideration that in the English-Angora linkage tests a difference of an opposite character was observed between the sexes.

A LINKAGE TEST WITH RESULTS PROBABLY NEGATIVE.

Tests for linkage between Vienna-White and Angora, as yet incomplete, have yielded a particularly interesting result (1) because, provisionally, they placed all known factors for white spotting in rabbits in one and the same linkage group, which would include English, Dutch, Vienna-White, and Angora. It is true that adequate tests had not yet been made for linkage between English and Vienna-White and between Dutch and Vienna-White, but since it is certain that both English and Dutch are linked with Angora, if Vienna-White is also linked with Angora, then English, Dutch and Vienna-White should be linked with each other. The matter will be tested by further experiments. Meanwhile, it will suffice to present the evidence already obtained as to the linkage relations between Vienna-White and Angora.

In the F_2 generation, from a cross between my original Vienna-White male and self-Angora females, several double recessive (Vienna-White Angora) individuals were obtained. One of these, a male, 6070, was mated with F_1 doubly heterozygous females, and has produced, up to the time of writing, over 50 litters of back-cross young, which have been reared to an age when they could be classified as to hair-length. A smaller number of young has been produced by a reciprocal back-cross. The character of the young is shown in Table 20. The total number is 439, of which 208, or 47.3 ± 1.6 per cent, are Angoras, while 216, or 49.2 ± 1.6 per cent are Vienna-Whites. These percentages agree sufficiently well (considering the size of the probable error) with the 50 per cent expected in each case. But when we consider the relation of Vienna-White to Angora among the back-cross young, we observe that considerably *less* than half the Vienna-Whites are Angora, while considerably *more* than half the colored young are such. This indicates linkage, the cross-over classes being colored short and Vienna-White Angora. Together they comprise 145 of the 439 young, or 44.4 ± 1.6 per cent. This is a deviation from 50 per cent of 5.6 per cent, or 3.5 times the probable error. It is not beyond question a statistically significant difference, although the probability of its occurrence as a chance event is only 1.8 in 100.

To see whether the linkage tendency had been manifested steadily throughout the back-cross experiment, I at one time divided the 46 litters, which up to that time had been recorded, as nearly as possible into four equal groups, in the order in which they were recorded. These are summarized separately in the first four entries of Table 20. Each group showed fewer cross-overs than non-cross-overs, the percentages ranging from 39 to 47. Since that time additional litters have been recorded, as indicated in the last two entries of Table 20. In all except the last of these partial summaries, non-cross-overs are in excess of cross-overs. It seems possible, therefore, that a genuine linkage exists, although its strength is low, being only 10.1 on a scale of 100, as indicated by the experiments up to the present time. But against the idea that linkage occurs between Vienna-White and Angora may be mentioned the fact that no linkage has been found to exist between English and Vienna-White. Since English and Angora are linked with each other, each should be linked with the same third character. Therefore, if Vienna-White is linked with Angora, it should also show linkage with English, but it does not. F_1 double heterozygotes produced by crossing English with Vienna-White have been back-crossed with Vienna-Whites (double recessives). Such matings have produced 83 colored young and 81 Vienna-Whites, a close approach to the expected 1 : 1 ratio. Of the 83

TABLE 20.—*Character of the back-cross young obtained in crosses of double recessive, Vienna-White Angora rabbits, with F_1 doubly heterozygous ones.*

	Colored short.	Colored Angora.	V. Wh. short.	V. Wh. Angora.	Percentage cross-overs.
Litters 1–12	17	21	19	17	45.9 ± 3.9
Litters 13–24	20	19	18	14	47.8 ± 4.0
Litters 25–36	16	20	26	15	40.2 ± 3.8
Litters 37–46	16	18	19	8	39.3 ± 4.3
Litters 47–60	14	21	22	17	41.9 ± 3.9
Litters 61–77	22	19	22	19	50.0 ± 3.7
Totals:	105	118	126	90	44.4 ± 1.6

colored young, 39 were English and 44 non-English, equality being expected if no linkage exists. The non-English young are cross-overs, and since they exceed the non-cross-overs (as 44 : 39), no linkage is indicated. The deviation from equality is 3 per cent, which is less than the probable error, 3.7 per cent. Accordingly it seems doubtful whether linkage exists between Angora and Vienna-White.

LINKAGE TESTS WITH RESULTS CERTAINLY NEGATIVE.

In Table 21 is given the complete list of linkage tests made, of which only those already described have yielded positive results. Those yielding negative results are equally valuable, though less interesting. They must now claim our attention. We shall take them up in the order given in Table 21.

Table 21.—*Summary of all back-cross linkage tests made.*

Genes.	Number of young.	Percentage cross-overs.	Probable error.	$\frac{\text{Diff.}}{\text{P. E.}}$	Remarks.
A-B	....	....	....	....	Covered by A-C.
A-C	110	46.4	3.2	1.12	
A-D	145	51.7	2.4	0.61	
A-E	128	46.8	2.9	1.04	
A-En	184	44.5	2.5	2.20	
A-L	....	....	....	....	Covered by A-En.
A-V	....	....	....	....	In progress.
B-C	1000	34.6	1.0	32.33	
B-D	....	....	....	....	Covered by B-C and C-D.
B-E	118	50.0	3.1	0	
B-En	173	53.7	2.5	1.44	
B-L	....	....	....	....	Covered by C-L.
B-V	136	55.1	2.9	1.76	
C-D	120	51.6	3.0	0.54	
C-E	83	58.7	3.7	2.29	
C-En	112	47.3 or 52.7	3.2	0.85	
C-L	170	55.2	2.6	2.00	
C-V	23	52.1	7.0	0.30	
D-E	252	50.4	2.1	0.18	
D-En	549	51.1	1.4	0.82	
D-L	....	....	....	....	Covered by D-En.
D-V	142	42.9	2.8	2.50	
E-En	272	50.7	2.0	0.35	
E-L	235	54.0	2.2	1.81	
E-V	35	42.8	5.7	1.26	
En-L	1560	13.0	0.8	43.4	
En-V	83	53.0	3.7	0.81	
L-V	439	44.4	1.6	3.50	
L-Du	1213	14.2	0.9	37.2	

The linkage relation of A to B has not been tested directly but is indirectly covered by the next test, A-C, for B and C have been shown to be linked with each other. Accordingly, if A is not linked with C, it is improbable that it should be linked with B.

The test for linkage between A and C was made by the use of F_1 gray animals produced in the course of crosses between Himalayan albinos and

Flemish-Giant rabbits described in my 1922 paper. These F_1 animals were of the formula $AaCc^H$, and they were back-crossed with double recessive mates of the formulæ aac^Hc^H or aacc, these being respectively Himalayan and Polish albinos homozygous for a. In a total of 232 young there were produced 59 gray, 51 black and 122 albino young. Theoretically, these classes should be as 1 : 1 : 2, if no linkage exists. That it probably does not exist is shown by the following considerations. Half the young should be albinos, whether or not linkage exists. The observed number 122 is close to expectation, 116 ± 5.1. No attempt was made to distinguish Aa from aa albinos, which would have been an exceedingly tedious and time-consuming operation involving individual breeding tests. Consequently the albinos are disregarded in calculating the cross-over percentage. This is indicated by the relation of Aa (gray) to aa (black) individuals. The latter will have resulted from cross-over gametes (aC) produced by the F_1 parent, the former from non-cross-over gametes (AC). The total number of colored young being 110, it is expected that if no linkage exists, there will be 55 ± 3.54 of each sort, gray and black. The observed deviation is 4 which is 1.1 times the probable error—an insignificant deviation.

If one objects to discarding the albinos altogether, since it is unknown how many of them are potentially grays and how many potentially blacks, he may retain them and base the criterion of linkage on the observed deviation from a 1 : 1 : 2 ratio, which ratio is expected if no linkage exists. The tabulation will then be as follows:

	Classes of young.		
	Gray.	Black.	Albinos.
Expected	58	58	116
Observed	59	51	122
Difference	1	7	6
P. E. (in numbers)	4.45	4.45	5.14
$\frac{\text{Diff.}}{\text{P. E.}}$	0.2	1.5	1.1

It will be observed that on this basis also no class deviates significantly from expectation. Consequently there is every reason to believe that no linkage exists.

In testing for linkage between genes A and D, a cross was first made between a blue-gray rabbit (Ad) and a black (aD). The F_1 animals were now back-crossed with blue mates (ad), producing four classes of young as follows:

Gray. AD	Blue-Gray. Ad	Black. aD	Blue. ad
35	29	41	40

The total number of young is 145; of which 75 (the gray and the blue) are cross-overs. This is 2.5 more than the expected number. The P. E. for

145 young is 4.06. Hence the ratio, $\frac{\text{diff.}}{\text{P. E.}}$, is $\frac{2.5}{4.06}$, or 0.6, which is statistically not significant. Of course the mere fact that cross-overs are in excess of 50 per cent shows that no linkage exists, but the further indication that the amount of the deviation is no greater than random sampling might produce is also interesting.

The test of linkage between genes A and E was made by first crossing a black rabbit (aE) with a yellow one (Ae). The resulting doubly heterozygous F_1 gray animals were next crossed with double recessive, sooty yellow (ae) mates, producing 128 young of four classes as follows:

Gray. AE	Black. aE	Yellow. Ae	Sooty. ae
31	39	29	29

The cross-over classes are here the gray and the sooty, which together number 60, which is 4 less than half the total. But as the probable error for 128 is 3.82, the ratio, $\frac{\text{diff.}}{\text{P. E.}}$, is $\frac{4.00}{3.82}$, or 1.04, a deviation from equality without statistical significance and hence not indicating linkage.

In order to test for linkage between genes A and En, a black English rabbit (aEn) was crossed with a gray self (Aen). The F_1 individuals were now mated with black self (double recessive) animals, producing 184 young as follows:

Gray English.	Gray Self.	Black English.	Black Self.
36	50	52	46

The cross-over classes are gray English and black self, and they are both smaller than the remaining (non-cross-over) classes, which suggests linkage. But is the difference a significant one? The total cross-overs are 82, which is 10 less than ½ n, 92. The P. E. for 184 young is 4.57. Hence $\frac{\text{diff.}}{\text{P. E.}} = \frac{10}{4.57} = 2.1$. A deviation as great as this might be expected 16 times in 100 through chance alone, accordingly the probability that it is due to linkage is small. Nevertheless this case calls for further investigation, either directly by repetition of the experiment, or indirectly by testing for linkage between A and L. Neither the A-L nor the A-V relations have yet been tested for linkage, though the former would presumably be negative in view of the negative result in case of A-En.

The test for linkage between B and C has already been discussed. Beyond question these two genes lie in the same linkage system.

B and E are shown to be independent of each other by a back-cross between doubly heterozygous females and a double recessive male. The male was a brown-eyed yellow individual (bbee) and his mates were black (or gray) individuals, heterozygous for both brown and yellow (BbEe). They had been produced by a cross of brown (bE) with yellow (Be). The back-cross matings produced 118 young of four classes as follows:

Black. BE	Brown. bE	Black-eyed yellow. Be	Brown-eyed yellow. be
30	33	26	29

The cross-over classes are black and brown-eyed yellow. Together they number 59, exactly equaling the non-cross-over classes, which also number 59. Hence no linkage is indicated by the experiment.

In order to test for linkage between genes B and En, a cross was first made between Brown-Self individuals and Black-English. The F_1 individuals were Black-English. These were now back-crossed with the Brown-Self variety (the double recessive) and four classes of young were obtained as follows:

Black-English.	Brown-English.	Black-Self.	Brown-Self.
40	43	50	40

The total number of young is 174. The cross-overs are Brown-English and Black-Self, together numbering 93. They are more numerous than the non-cross-overs which number 80. Obviously there is no linkage, the cross-over percentage being 53.7 ± 2.56. The deviation from 50 per cent is 3.7, which is 1.4 times the P. E., and explainable as due to chance alone.

In order to test for linkage between genes B and V, a cross was made between chocolate females and my original Vienna-White male. The F_1 young were gray coated. In F_2 the recessive Vienna-White character was recovered in individuals some of which had black eyes, others having brown eyes. One of the latter, ♂ 6091, homozygous for both recessive characters, b and v, was back-crossed with F_1 females and produced 136 young of the four classes:

Black. BV	Brown. bV	Black-eyed Vienna Wh. Bv	Brown-eyed Vienna Wh. bv
38	31	30	37

The first and last of these are the cross-over classes. Together they number 75, while the non-cross-over classes number 61, the total being 136. The cross-over percentage is in excess of 50, namely 55.1 ± 2.89. This shows that no linkage occurs. The departure from 50 per cent is 5.1 per cent, which is 1.7 times the probable error, explainable as due to chance alone (random sampling).

Back-cross tests for linkage between genes C and D were made with F_1 animals, some of which were so constituted as to show repulsion, while others were so constituted as to show coupling, if the genes under discussion lie in the same chromosome. The first-mentioned sort of F_1 animals was produced by a cross between a dilute pigmented animal and an albino transmitting the intensity factor; the second sort was produced by a cross between an intense pigmented animal and an albino transmitting dilution. Both sorts of F_1 animals were back-crossed with the double recessive variety (albinos homozygous for dilution). The repulsion crosses produced 78 colored young together with 83 albinos. It was impossible to tell by inspection of the albinos whether or not they carried intensity, so they were discarded and the test for linkage was based wholly on the colored young. Of these, 42 were intense (cross-overs) and 36 dilute (non-cross-overs). If no linkage exists, half the young, or 39 are expected in each class. The observed departure from 39 is 3, which is scarcely more than the probable error for 78 young, namely 2.98. Hence there is no indication of linkage quite apart from the fact that cross-overs are in excess of non-cross-overs, itself a decisive argument against the occurrence of linkage.

In the coupling test there were produced 42 colored young and 38 albinos. Disregarding the latter, there were among the colored young 22 intense and 20 dilute, the latter being cross-overs. Exact equality of the two classes would place 21 in each. The observed departure from this is 1, but as the probable error for 42 young is 2.2 individuals, the departure is less than the P. E. and so not significant.

Combining the coupling and repulsion series, we have, in a total of 120 colored young, 62 cross-overs, which is 2 more than half the total number, a departure less than the P. E., 3.7. Consequently the evidence against the occurrence of linkage is ample and conclusive.

In preparation for a test for linkage between genes C and E, a cross was made between a yellow individual (Ce) and albinos transmitting black (cE). Several F_1 females (CeEe), which were gray in appearance, were now back-crossed with an albino male (4408) known from his parentage to be a double recessive (ce), since both his parents were yellow. These matings produced 95 albino young and 83 colored ones. Disregarding the former, we may note that of the colored young 49 were black (cross-overs) and 34 yellow (non-cross-overs). The fact that cross-overs are in excess indicates that no linkage occurs. The cross-over percentage is 58.7 ± 3.7, a deviation 2.3 times the probable error, but undoubtedly due to random sampling, not to linkage. Comparison should be made in this connection with the result of the A-En test, where the deviation was less than in this case, and to be explained therefore as probably due to random sampling rather than linkage.

A test for the existence of linkage between genes C and En was made by mating ordinary albino females, which did not transmit the English pattern, to an English male, 2053, heterozygous both for English and for albinism. It is uncertain whether he inherited English in the same gamete with albinism or not, since both his parents were colored and it is probable that both were heterozygous for albinism, but for our present purpose this is immaterial. One of his parents was English, the other Dutch of grade 15. In the test he was back-crossed with albino females and produced three classes of young, (1) English, (2) Dutch (or self), and (3) albinos. In the case of the albino young it would be uncertain without breeding tests whether an individual carried English or Dutch, so no attempt was made to classify them. They are simply disregarded. The albino young should be half of the total whether or not linkage exists. In fact they were 111, the colored young being 112, of which 53 were English and 59 Dutch (or self). It is uncertain whether the English or non-English group represent the cross-overs, as already stated, but in either case no linkage is indicated. For without linkage it is expected that half the young will be cross-overs. Half of the 112 colored young is 56, and the deviation of each of the observed classes from this is 3. But the P. E. for 112 young is 3.5. Therefore the deviation is less than the P. E. and gives no indication of linkage. The cross-over percentage is either 47.3 or 52.7 ± 3.19.

In testing for linkage between albinism and Angora coat (genes C and L), F_1 individuals were for the most part used which had resulted from a cross between a short-haired colored individual and a long-haired albino, but a few were also employed, in the production of which a colored Angora had been crossed with an albino having short hair. The first sort of F_1 animals will be called *repulsion*, the second sort *coupling* animals. Both sorts were back-crossed with double recessive mates, albino Angoras.

The repulsion crosses produced young of four sorts as follows:

Colored short.	Colored Angora.	Albino short.	Albino. Angora.
42	23	38	36

The cross-over classes are colored short and albino Angora, together numbering 78, while the non-cross-over classes number 61, the total being 139. As cross-overs are in excess of non-cross-overs, there is no evidence here for linkage. The coupling crosses produced 31 young as follows:

Colored short.	Colored Angora.	Albino short.	Albino Angora.
6	10	6	9

The two middle classes are here the cross-overs, numbering 16, as against 15 non-cross-overs. Again there is no suggestion of linkage.

Combining both series of crosses, we have, in a total of 170 young, 94 cross-overs, which exceeds half the total number by 9. This is 2.0 times the P. E. for 170 young, which is 4.40. But this is not a deviation greater than chance alone might produce, and as cross-overs are in excess of non-cross-overs there is strong evidence against the occurrence of linkage.

For testing the linkage relation between genes C and V, a cross was made between chinchilla and Vienna-White. The F_1 individuals were gray with white (Dutch like) markings characteristic of individuals heterozygous for Vienna-White. In F_2 were obtained gray, chinchilla, and Vienna-White classes. With the idea of securing as speedily as possible Vienna-White individuals homozygous for chinchilla, F_2 chinchilla individuals were selected which indicated by their white markings that they were heterozygous for Vienna-White. Mating individuals of this sort produced in F_3 Vienna-White individuals which would certainly be homozygous for chinchilla, the desired double recessive combination. These have been mated to F_1 double heterozygotes and have produced 6 litters of back-cross young of the following classes:

Full color.	Chinchilla.	Vienna-White.
12	11	16

No attempt has been made to differentiate Vienna-Whites carrying full color from those which carry chinchilla. They are indistinguishable in appearance. Consequently the other two back-cross classes must serve as the test for linkage. Those showing full color are the cross-overs, the chinchillas being non-cross-overs. Numerically they are as 12 to 11, as nearly equal as is possible in an odd number of individuals. Since cross-overs are in excess, it seems doubly improbable that linkage exists. The deviation from equality is 0.5, where the P. E. is 1.6, so the deviation is without significance and we may safely conclude that the genes in question are independent of each other (located in different chromosomes).

In preparing to make a test for linkage between genes D and E, intense black individuals were crossed with dilute yellow ones. The F_1 individuals were intense and black in pigmentation but of course were heterozygous for the alternative recessives, dilution and yellow. They were now back-crossed with double recessives, dilute yellows, producing 252 young as follows:

Intense black.	Dilute black.	Intense yellow.	Dilute yellow.
84	53	74	41

The cross-over classes are dilute black and intense yellow. Together they number 127, the non-cross-overs numbering 126. Here is strong evidence against the existence of linkage for the cross-overs and non-cross-overs are as nearly equal as possible. The deviation is only a fraction of the probable error.

When I first began the study of the linkage relation of genes D and En, I obtained indications of a positive association between them. In 1920, I reported on a back-cross which had produced, in a total of 83 young, 32 cross-overs, or 38.5 ± 3.7 per cent, a deviation from equality 3.1 times as great as the P. E. This result was obtained from a single F_1 male animal back-crossed with double recessive females. However, when later I made use of other F_1 animals, I obtained very different results without indication of linkage. Either the result obtained in the first case was due to deceptive random sampling or else the male in question was of peculiar genetic constitution. I have not since found an F_1 animal which gave a like result, though I have tested two others extensively. Unfortunately the first animal was already dead, so no further tests could be made with him. His complete record is given herewith (see ♂ 2123). In all he produced 88 back-cross young of which 35 or 39.7 ± 3.59 per cent were cross-overs. This is a deviation 2.8 times the P. E.

But the other two males have produced a slight excess of cross-over gametes which more than offsets the deficiency shown by 2123. Consequently when the records for all three are combined, there are 549 back-cross young, giving a cross-over percentage of 51.1 ± 1.44. The deviation from 50 per cent is now only 0.82 of the P. E. and so of no significance. We may conclude with considerable assurance that English and dilution are in general not linked.

If dilution is not linked with English, it is improbable that it would be found to be linked with Angora, since that is known to be linked with English. Accordingly no test for D-L linkage has been made.

My original Vienna-White male was homozygous for dilution. All his colored young which were intense in their pigmentation were accordingly doubly heterozygous for D and V. A back-cross with the original male or with Vienna-White individuals of similar constitution affords a satisfactory test for linkage between D and V. Matings of this sort have produced 287 young, of which 145 were Vienna-White and 142 colored. The latter alone can be classified as intense or dilute. Of them 61 were dilute (cross-overs) and 81 intense (non-cross-overs). The cross-over percentage is 42.9 ± 2.8. The departure from 59 per cent is two and a half times the P.E. and probably not significant.

The linkage relation of E to En has been tested with results emphatically negative. The test has been made chiefly with an F_1 male, 4393, which was also used extensively in the D-En tests, many of the same litters of back-cross young entering into both sets of experiments. The mother of this male was a Black-English rabbit, the father was a dilute yellow. He was accordingly heterozygous for English, agouti, and dilution. Apart from the tests which his back-cross young afford, there are included in the E-En linkage tests only 5 litters of young (22 individuals); these were produced by F_1 females. They make up a small part only of the total 272 young included in the tests. Disregarding the presence or absence of the agouti and intensity factors, the young may be classified as follows:

TABLE 22.—*Back-cross tests for linkage of dilution and English.*

F_1 male.	English intense.	English dilute.	Non-Eng. intense.	Non-Eng. dilute.	Total.	Cross-over percentage.	Diff./P. E.
2123...	23	17	18	30	88	39.7 ± 3.59	2.8
4393...	49	58	51	52	210	41.9 ± 2.33	.81
4595...	60	66	71	54	251	54.1 ± 2.13	2.51
Totals:	132	141	140	136	549	51.1 ± 1.44	.82

Black English.	Black Self.	Yellow English.	Yellow Self.
68	63	75	66

The two middle classes are the cross-overs. Together they number 138 young, or 50.7 ± 2.04 per cent of the total. The deviation from 50 per cent is only a fraction of the P. E. and obviously referable to random sampling alone. Consequently there is no indication of linkage.

Tests for linkage between genes E and L (yellow and Angora) were made by back-crosses of F_1 animals of two sorts, which will be called "repulsion" and "coupling" animals respectively. In the production of the former, E and L entered the cross in different gametes, in the case of the latter they entered together. The following classes and numbers of young were produced in the back-crosses.

The outside classes are cross-overs in the repulsion series, the middle classes in the coupling series. In both series cross-overs are slightly in excess of non-cross-overs, but the deviation from equality is less than twice the P. E. Both facts are evidence that no linkage exists.

TABLE 23.—*Back-cross young produced in tests for linkage between genes E and L.*

	Short black.	Angora black.	Short yellow.	Angora yellow.	Total.	Cross-overs.	Percentage cross-overs.	$\frac{\text{Diff.}}{\text{P. E.}}$
Repulsion..	23	21	21	27	92	50	54.3±3.52	1.2
Coupling ..	39	37	40	27	143	77	53.8±2.82	1.3
Total:					235	127	54.0±2.20	1.8

A small series of tests has been made for linkage between E and V (yellow and Vienna-White). It consists of 10 litters of young produced by F_1 females of the "repulsion" sort, mated with double recessive males (Vienna-White homozygous for yellow). Unfortunately, my double recessive males (father and son) both died before the tests had been completed, but the results obtained, as far as they go, indicate that no linkage exists. The back-cross young number 65, but 30 of them are Vienna-Whites which can not be classified as carriers of black or yellow respectively, so they must be disregarded. There are left 35 young, 15 of which are cross-overs (black) and 20 of which are non-cross-over (yellow). The cross-over percentage is 42.8 ± 5.7. The deviation from 50 per cent is 1.2 times the P. E., which is probably not significant. Provisionally we may conclude that no linkage exists.

The remaining gene relations listed in Table 21 have already been discussed.

SUMMARY.

1. Chinchilla in rabbits is shown to be an allelomorph of albinism, similar to the chinchilla variation in mice, the ruby-eyed dilute variation of rats, and the silver-gray variation of guinea-pigs. In this variation the activity of the color factor scarcely reaches the threshold for the production of yellow pigment, though black pigment is produced in nearly full amount.

2. Two different grades of chinchilla are described, one "dark," the other "pale." These are allelomorphs of each other as well as of all the previously known members of the albino allelomorph series in rabbits. The entire series as now known, in the order of dominance of its members, is (1) full color, (2) dark chinchilla, (3) pale chinchilla, (4) Himalayan albinism, (5) complete albinism. Each is shown to be a variant of the same genetic locus by its similar linkage relation to black vs. brown pigment.

3. The genetic combination, yellow chinchilla, produces a white rabbit with gray eyes, since the gene for chinchilla makes impossible the development of any appreciable amount of yellow pigment. This variety resembles Vienna-White, but is very different from it in its factorial constitution.

4. The Vienna-White variety has snow-white fur and blue eyes. The blue of the eye is due to lack of pigment in the front wall of the iris, like the blue eye of man. This is a recessive variation. Its gene is probably not linked with any other known gene of rabbits, although some indications were obtained of its linkage with Angora, but these are almost certainly negatived by the absence of linkage with English which is known to be linked with Angora.

5. With the exception of the albino series of allelomorphs, all factors in rabbits which produce white areas in the coat, or a coat generally white, act coöperatively, not antagonistically. Thus the recessive variations, Dutch- and Vienna-White, when crossed with the dominant variation, English, produce a whiter English, one with less extensive pigmented areas. When crossed with each other, they produce a whiter Dutch than either one crossed with self.

6. Neither Vienna-White nor Dutch is completely recessive in a cross with self. The heterozygote has in either case white markings of a Dutch type but much reduced as compared with a homozygous Dutch. The heterozygous Vienna-White has a "wall-eye," the front of the iris being usually in certain sectors only, but sometimes completely devoid of pigment. A similar wall-eye is frequently seen in homozygous Dutch rabbits, but rarely, if ever, in heterozygous Dutch.

7. The distinctness of Vienna-White from Dutch is seen not only in the differences in pattern produced both as homozygotes and as heterozygotes, but also in their very different linkage relations.

8. The white-spotting factors, English, Dutch and Vienna-White, differ fundamentally in nature from the factors of the albino series of multiple allelomorphs. Any cross between members of the two series produces an animal which develops full color in part or all of its coat. The two series are thus clearly complementary. All members of the albino series are variants of one genetic locus, the color factor. Those of the white spotting series are independent mutations of three different genetic loci. It happens that two of these (English and Dutch) lie in the same chromosome, the third (Vienna-White) lies in a different chromosome.

9. The factors Dutch, English and Angora are linked; their genes lie in one chromosome, probably in the order named. Only 0.1 per cent of crossing over has been observed between Dutch and English but each of these genes shows crossing-over with Angora in a frequency of 12 to 14 per cent.

10. The only other positively demonstrated linkage in rabbits is between the color-factor and its allelomorphs on one hand and black *vs.* brown on the other hand. Both these genes lie in the same chromosome and the crossover percentage between them is 34.6 ± 1.0.

11. No positive evidence has been obtained of a difference in rate of crossing-over between the two sexes in rabbits, though such a difference is known to occur in rats and mice.

12. The nine unifactorial characters of rabbits discussed in this paper, as indicated by their linkage relations, lie in 6 different chromosome pairs, only 2 of which contain more than one known gene.

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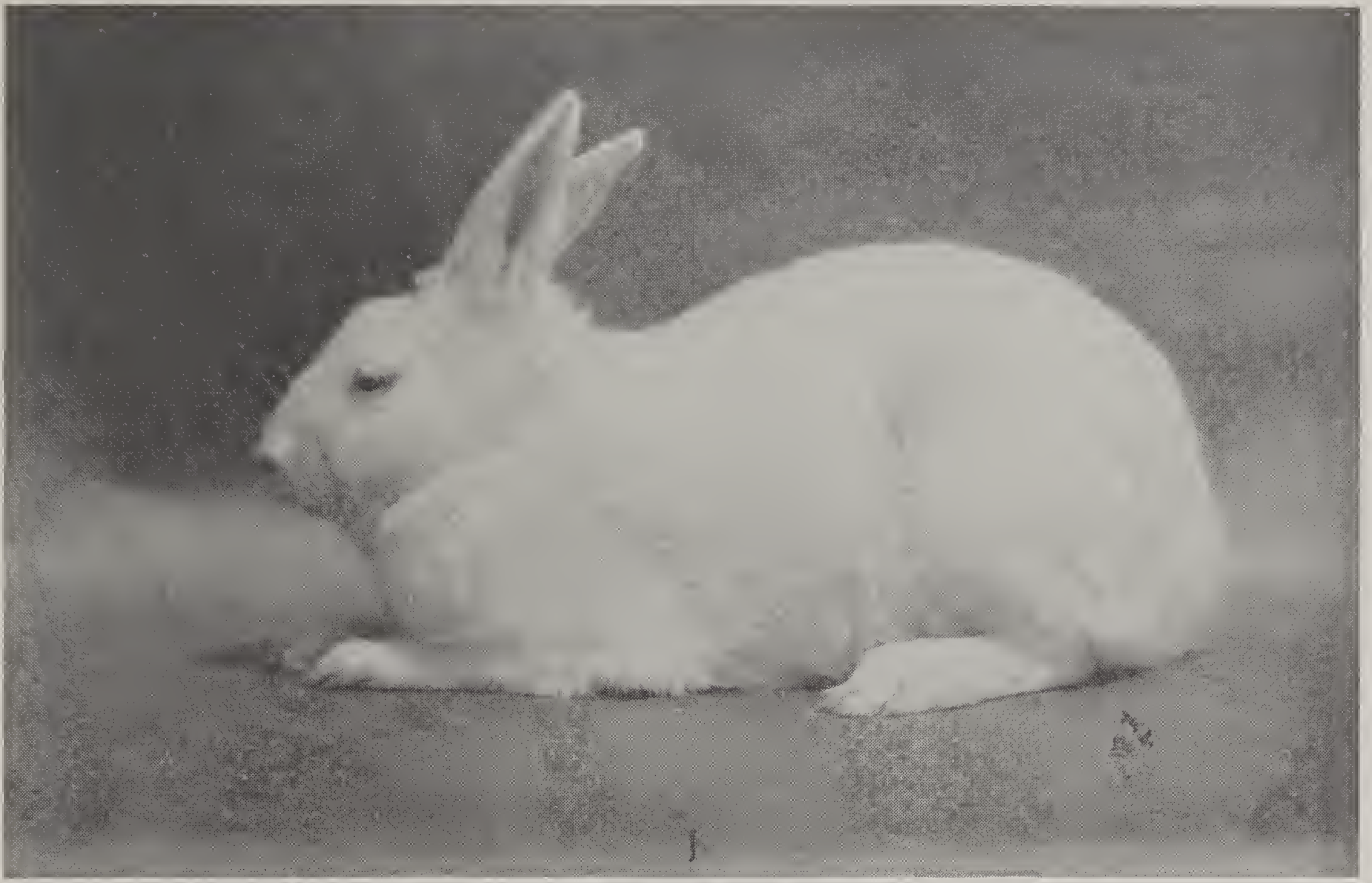

Fig. 1.—True or complete albino of the variety known as Polish.

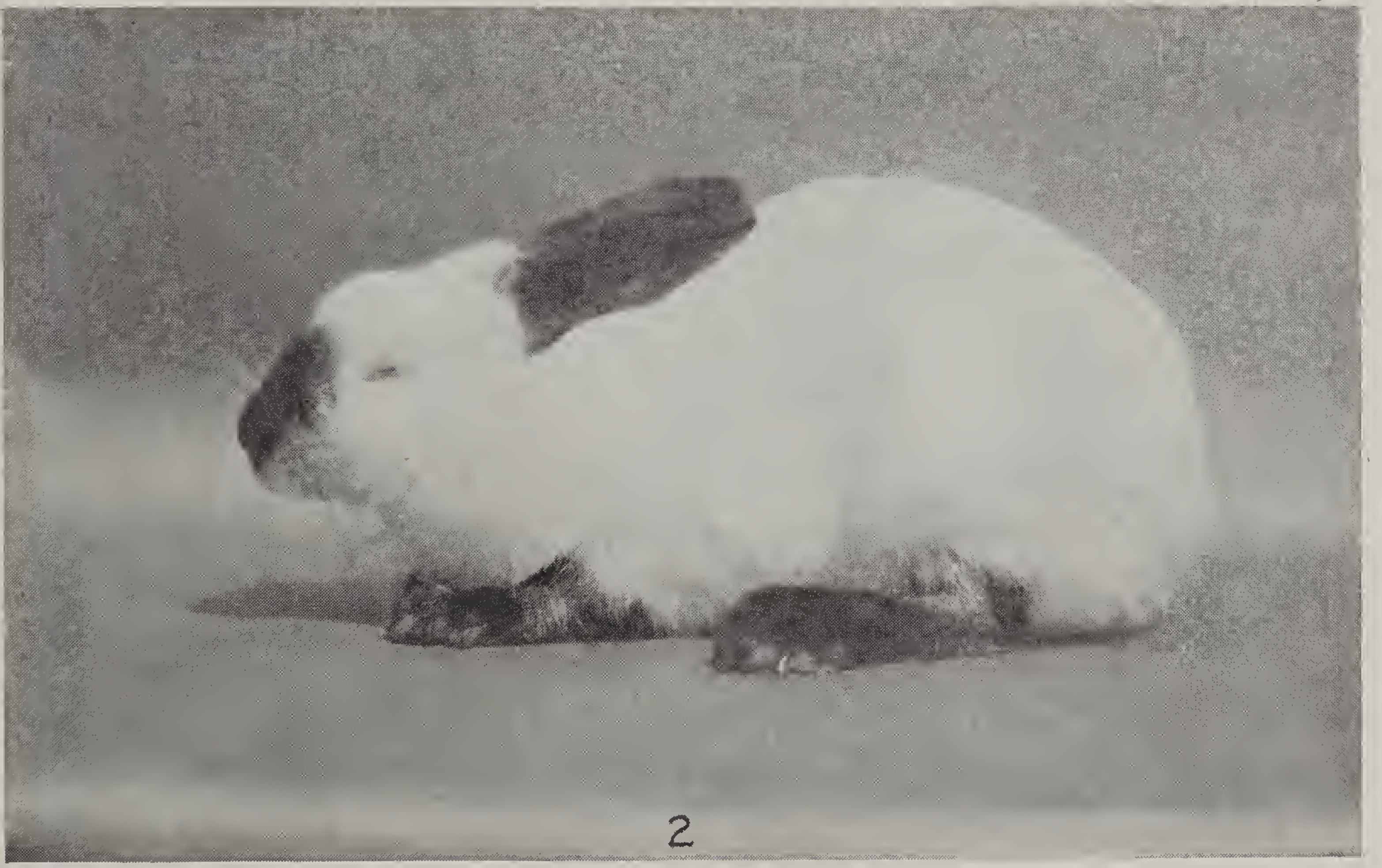

Fig. 2.—Himalayan albino.

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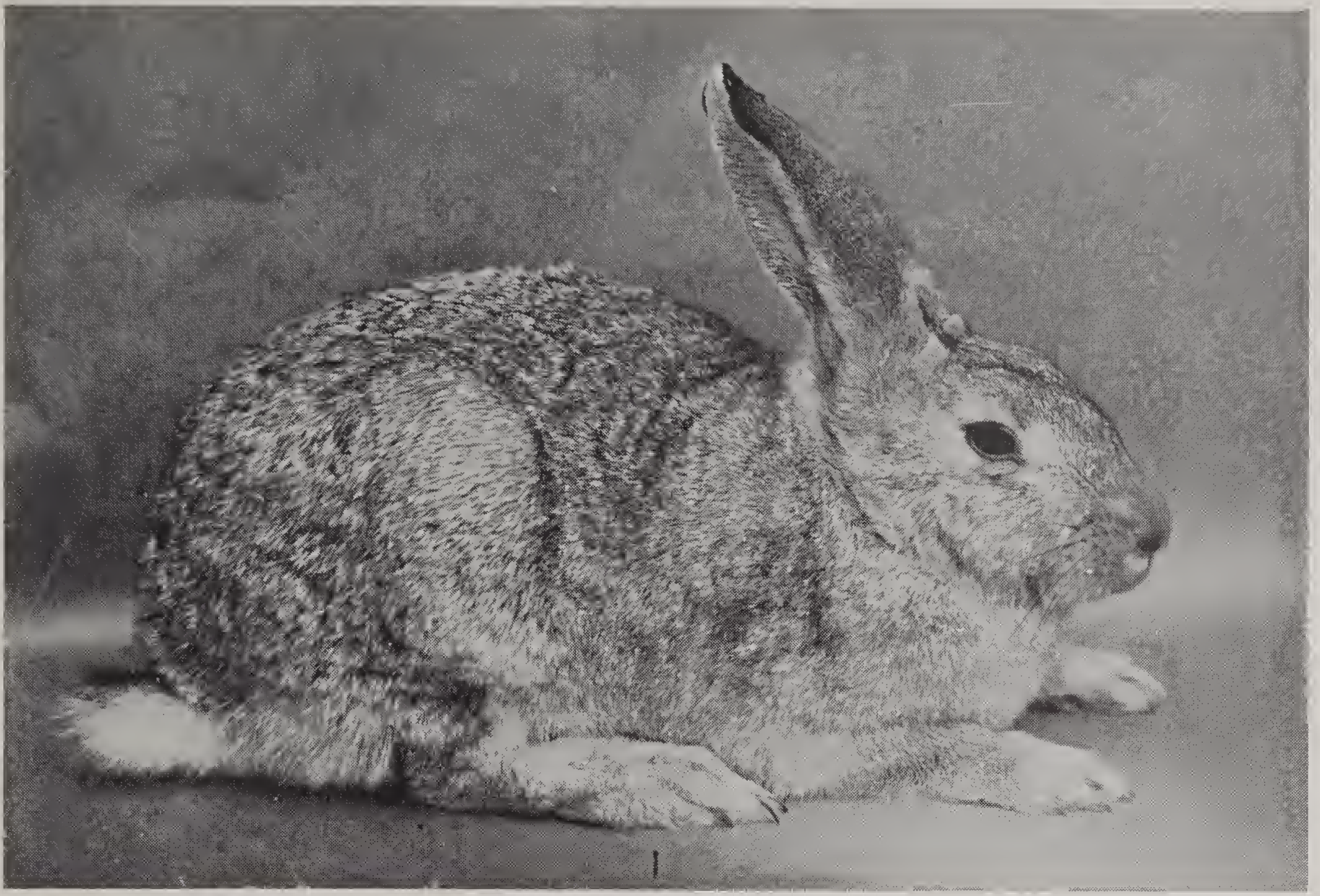

Fig. 1.—Pale chinchilla. This rabbit was a heterozygote between pale chinchilla and Himalayan albinism. Compare Plate 1, figure 2.

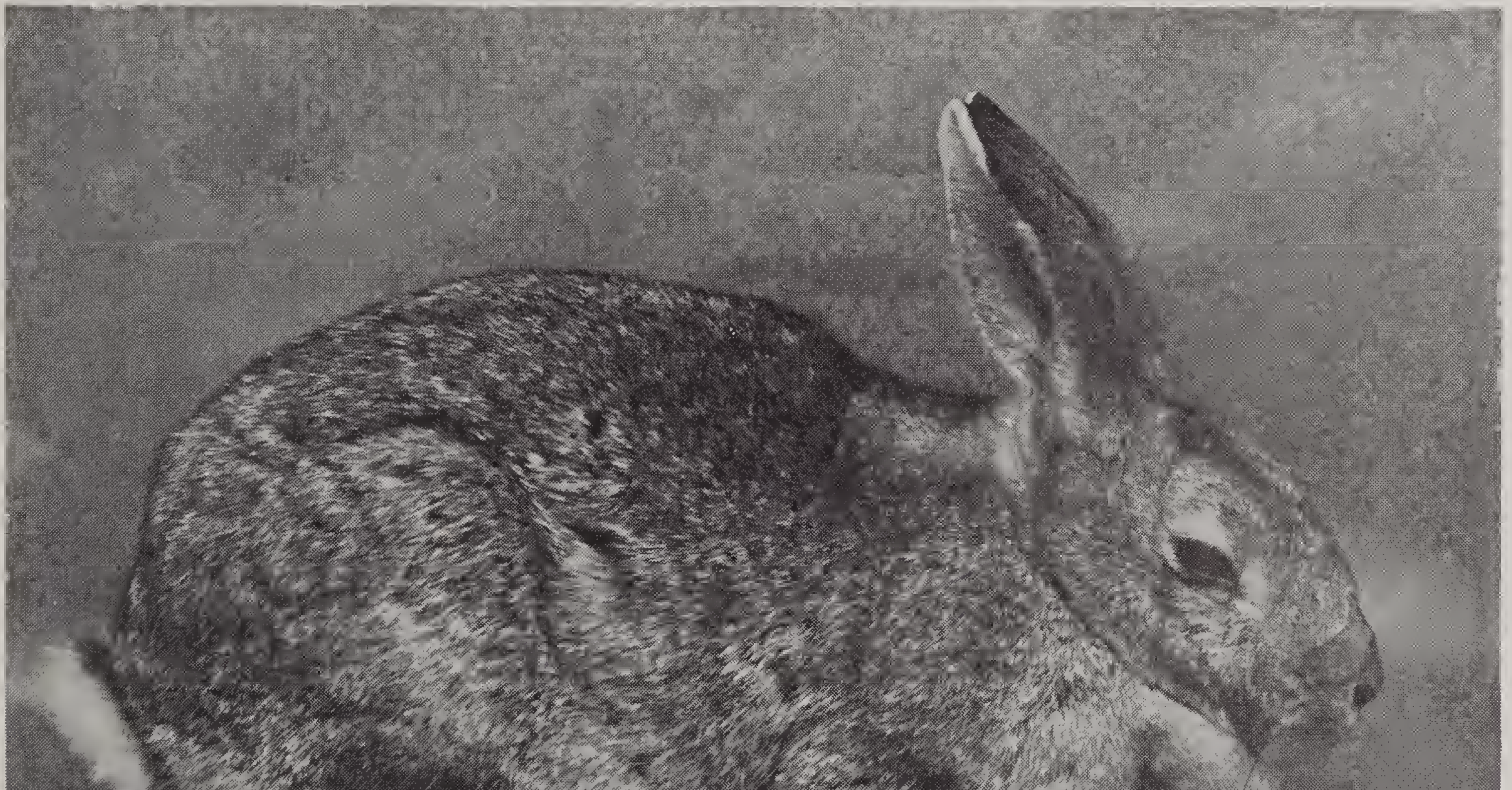

Fig. 2.—Dark chinchilla. This rabbit was a heterozygote between dark chinchilla and complete albinism. Compare Plate 1, figure 1.

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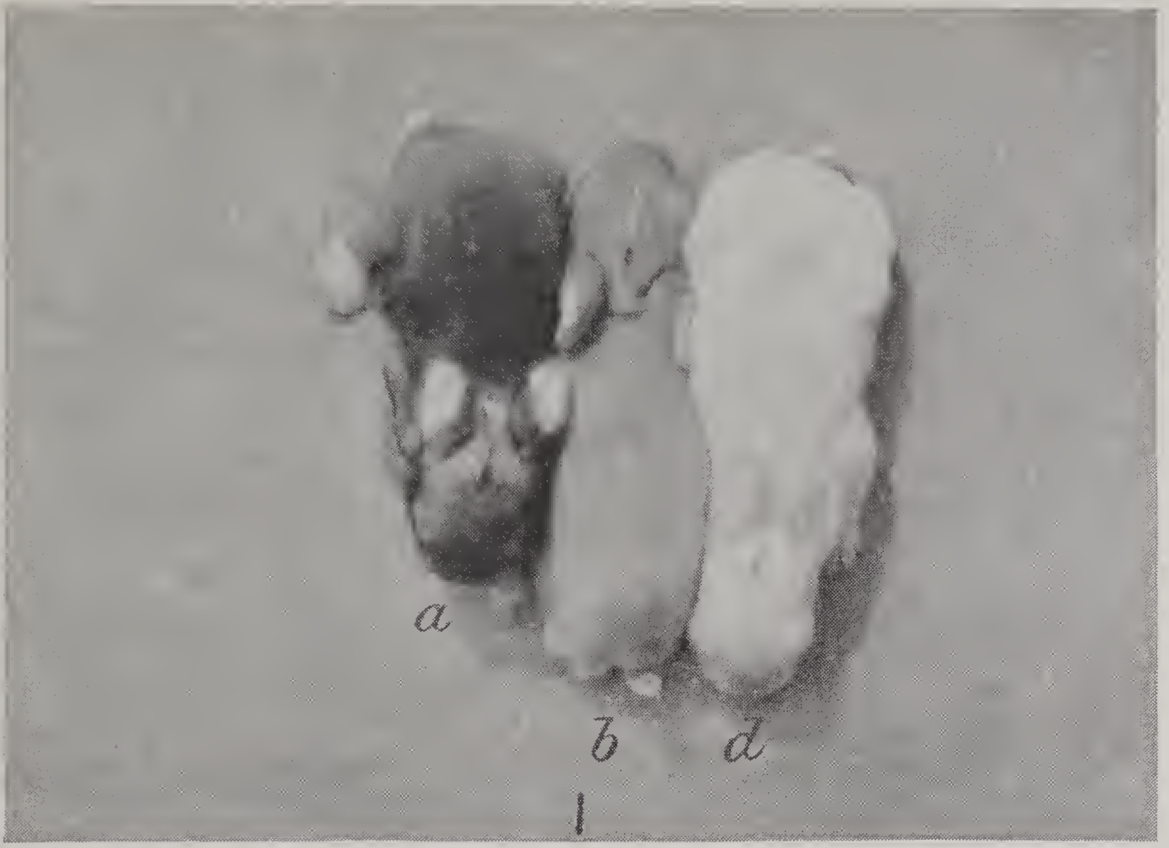

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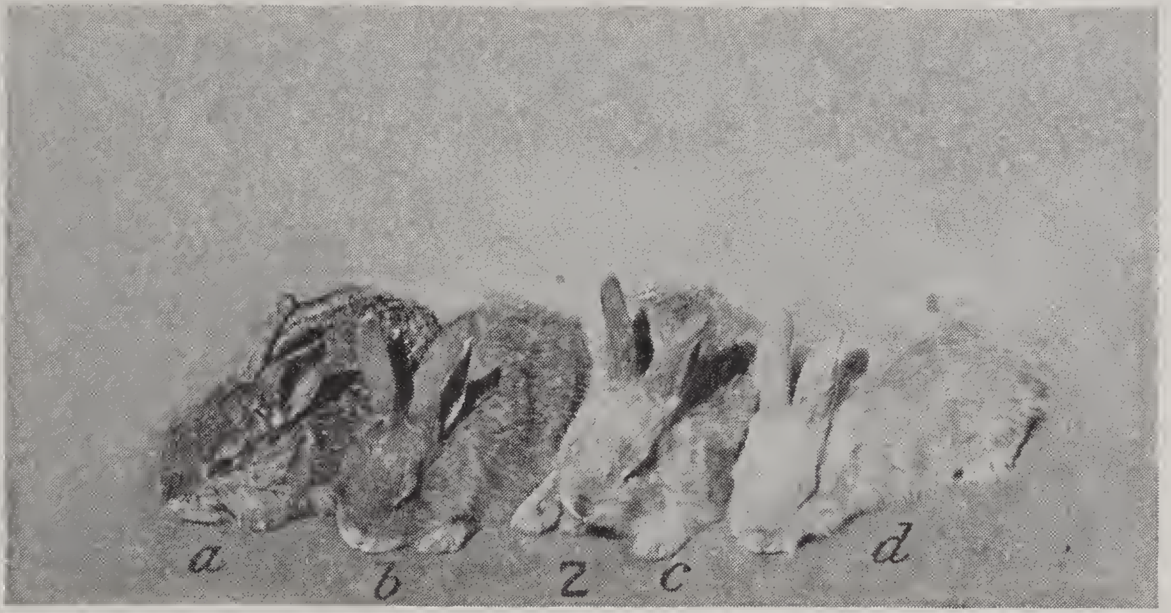

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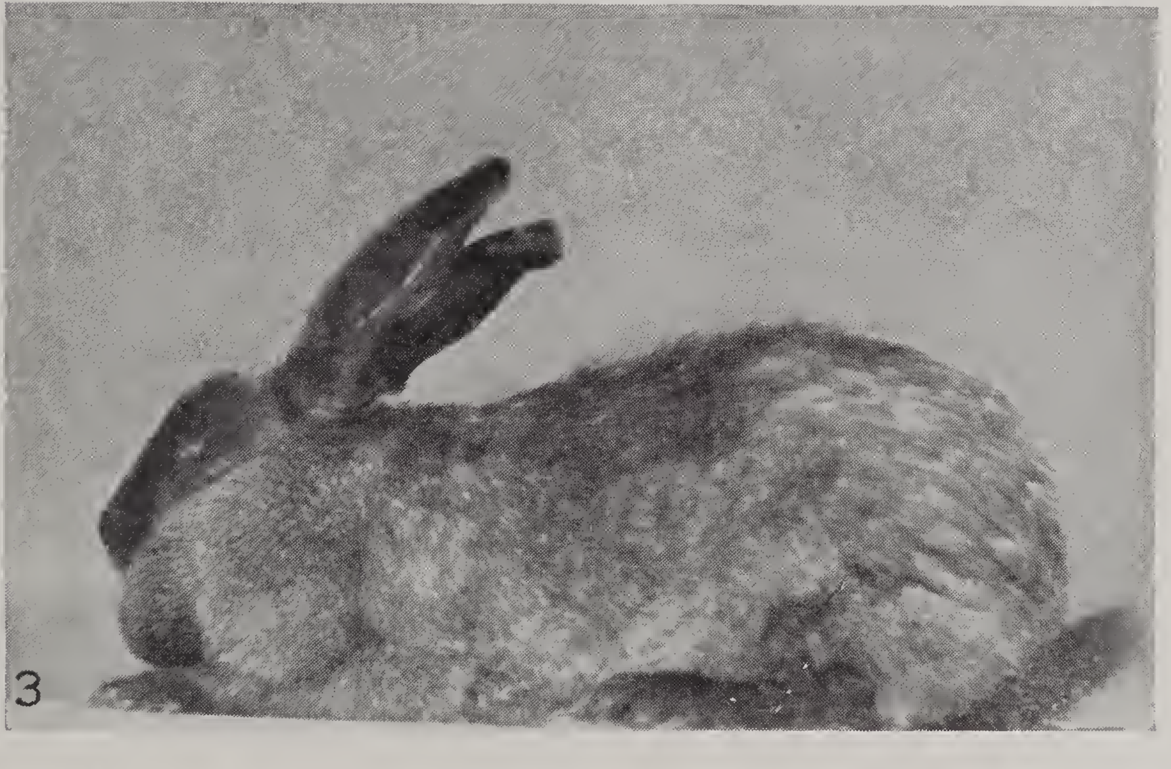

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Fig. 1.—Three young from a litter of chinchilla rabbits aged about ten days. The mother was a dark chinchilla heterozygous for pale chinchilla and also for blue dilution (an independent character). She was mated with an albino (Plate 1, figure 1) homozygous for dilution. *a*, Intense dark chinchilla; *b*, dilute dark chinchilla; *d*, pale chinchilla, probably dilute.

Fig. 2.—Same three young shown in figure 1, together with a fourth litter mate, photographed about ten days later. *a*, Intense dark; *b*, dilute dark; *c*, intense pale, *d*, dilute pale.

Fig. 3.—Non-agouti chinchilla or "sepia" rabbit.

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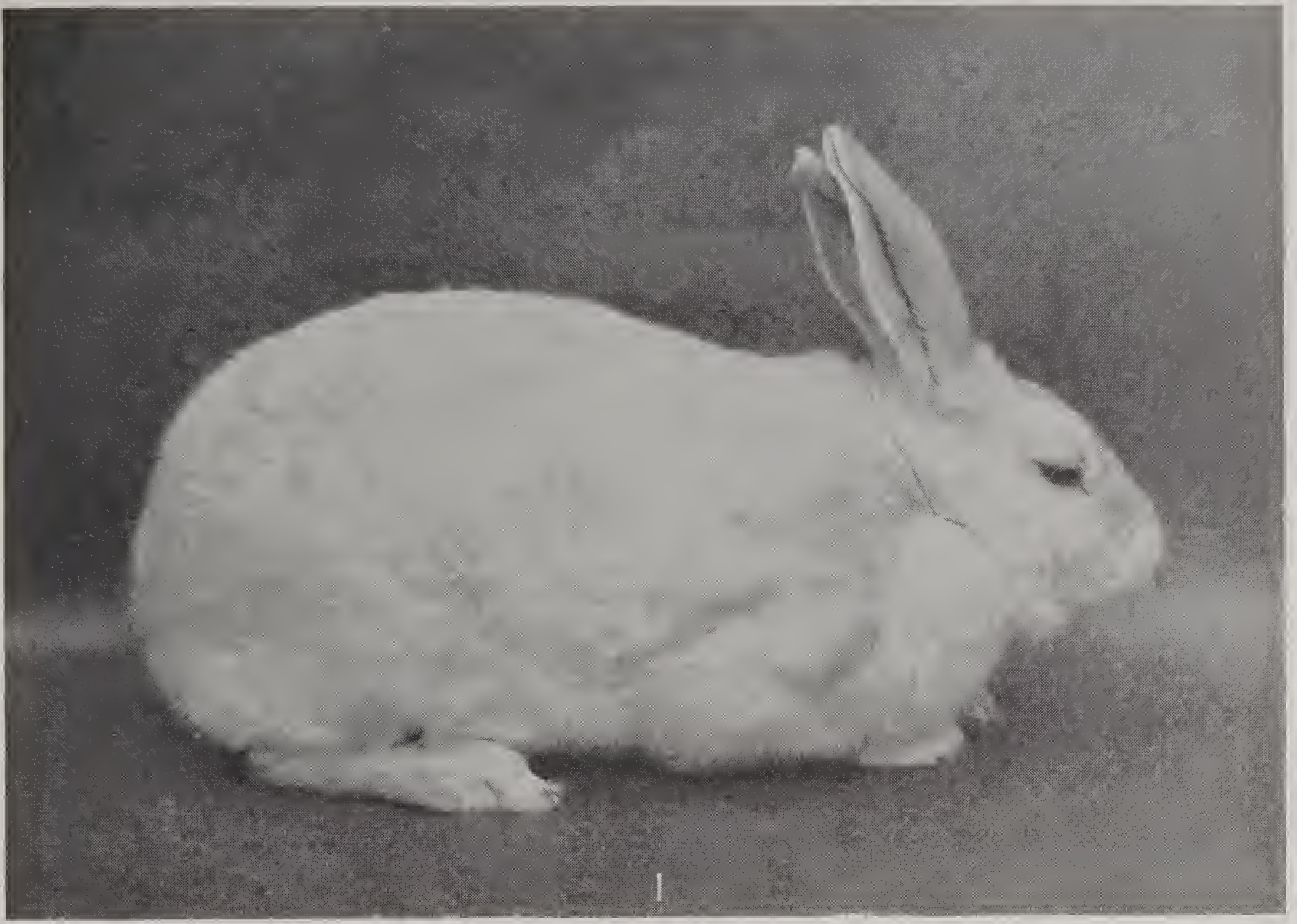

FIG. 1.—Genetically a "yellow chinchilla" rabbit, but its coat is white and its eyes gray.

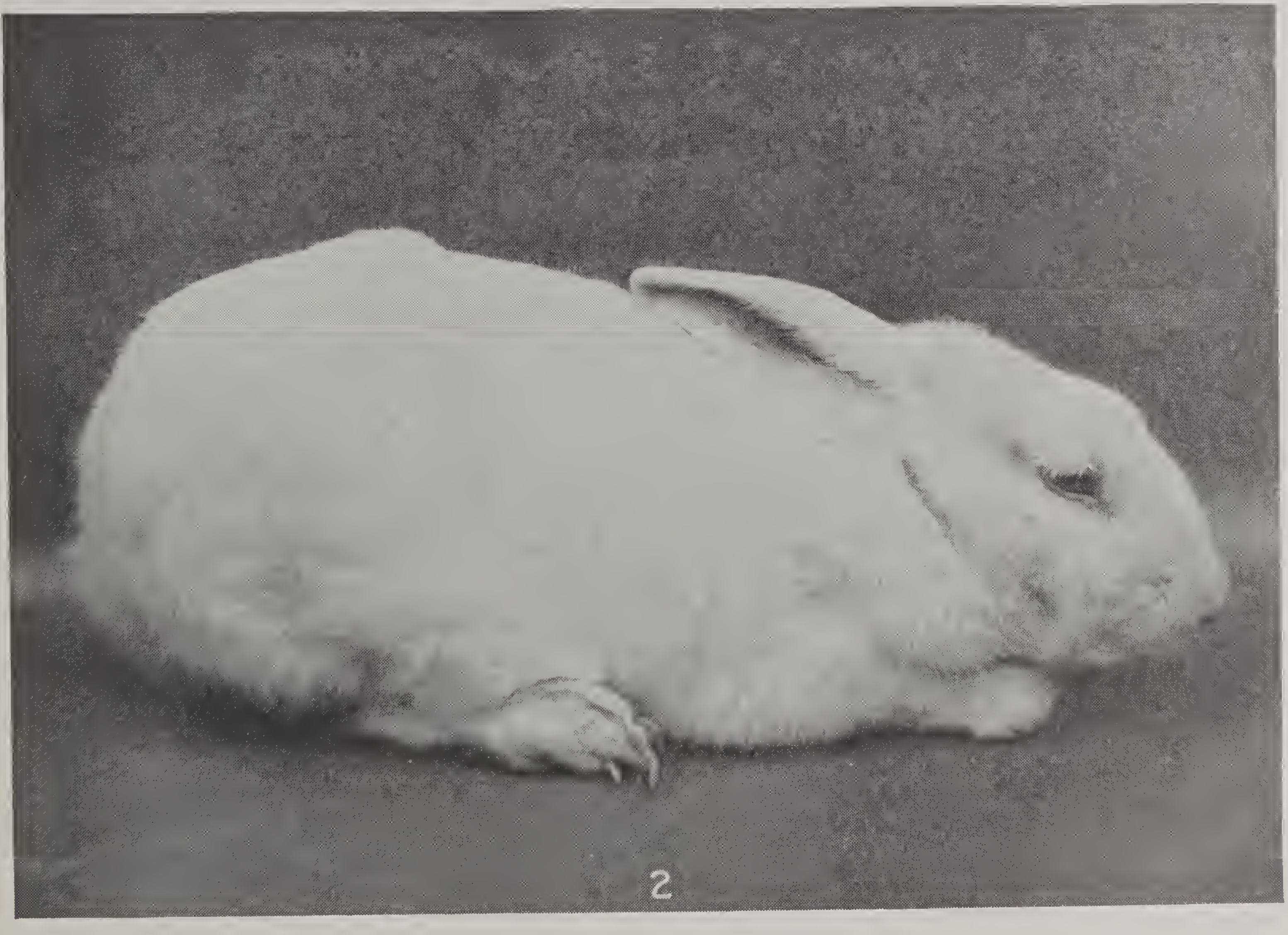

FIG. 2.—Original Vienna-White male from which entire experimental stock of this variety was descended. Coat white, eyes blue.

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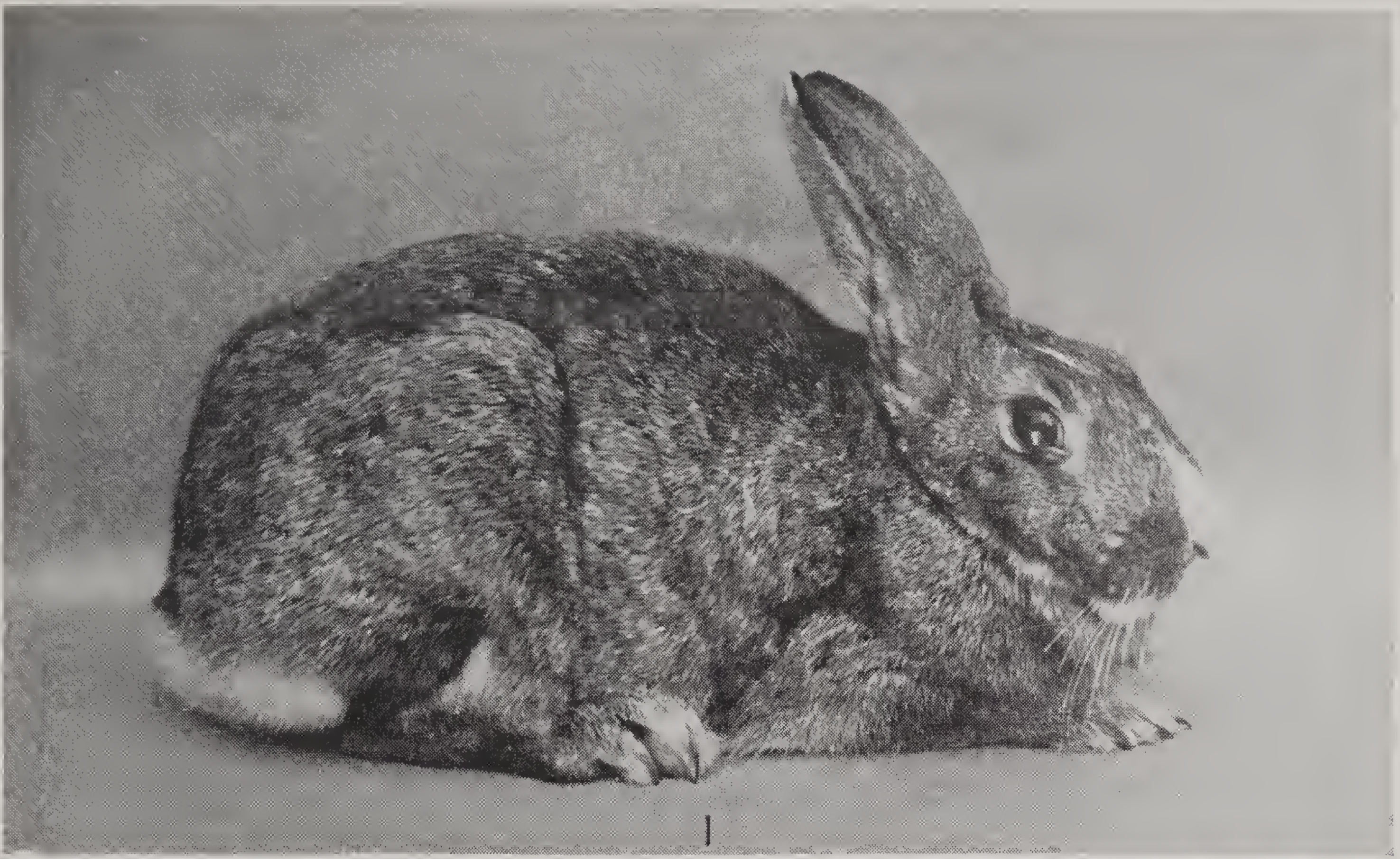
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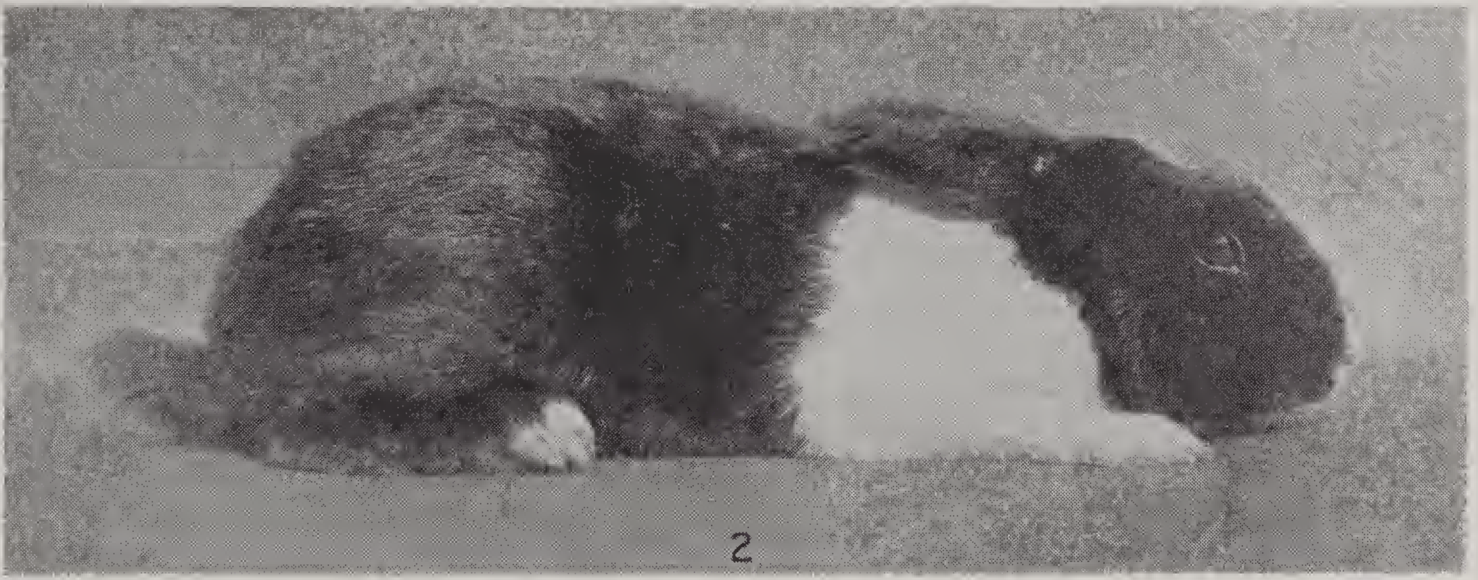
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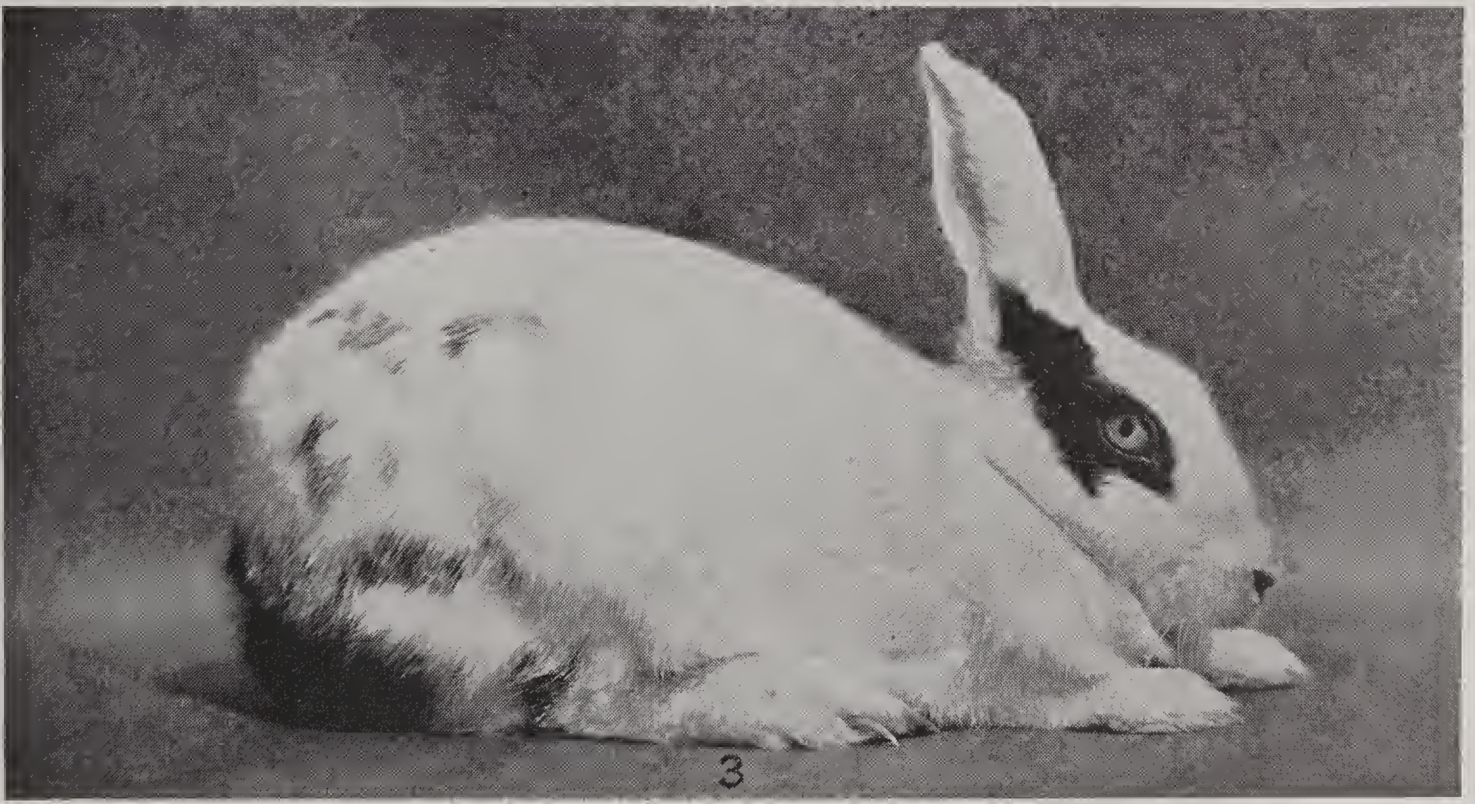
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Fig. 1.—One of gray F_1 offspring from crossing of two white varieties shown in Plate 4.
Fig. 2.—Homozygous Dark-Dutch rabbit.
Fig. 3.—Homozygous White-Dutch rabbit. Note whitish iris (wall-eye).

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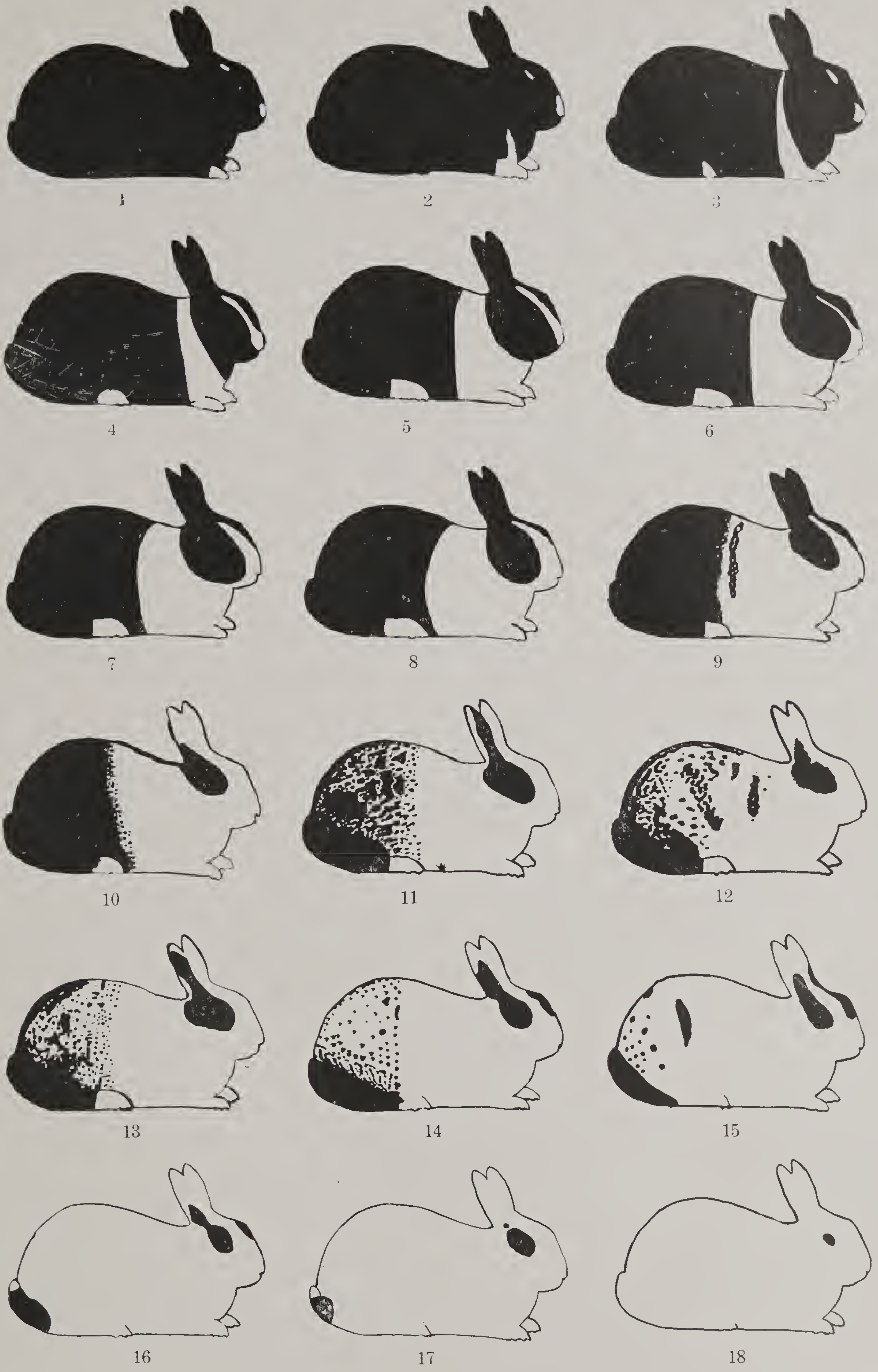

Grades 1 to 18 of Dutch rabbits.

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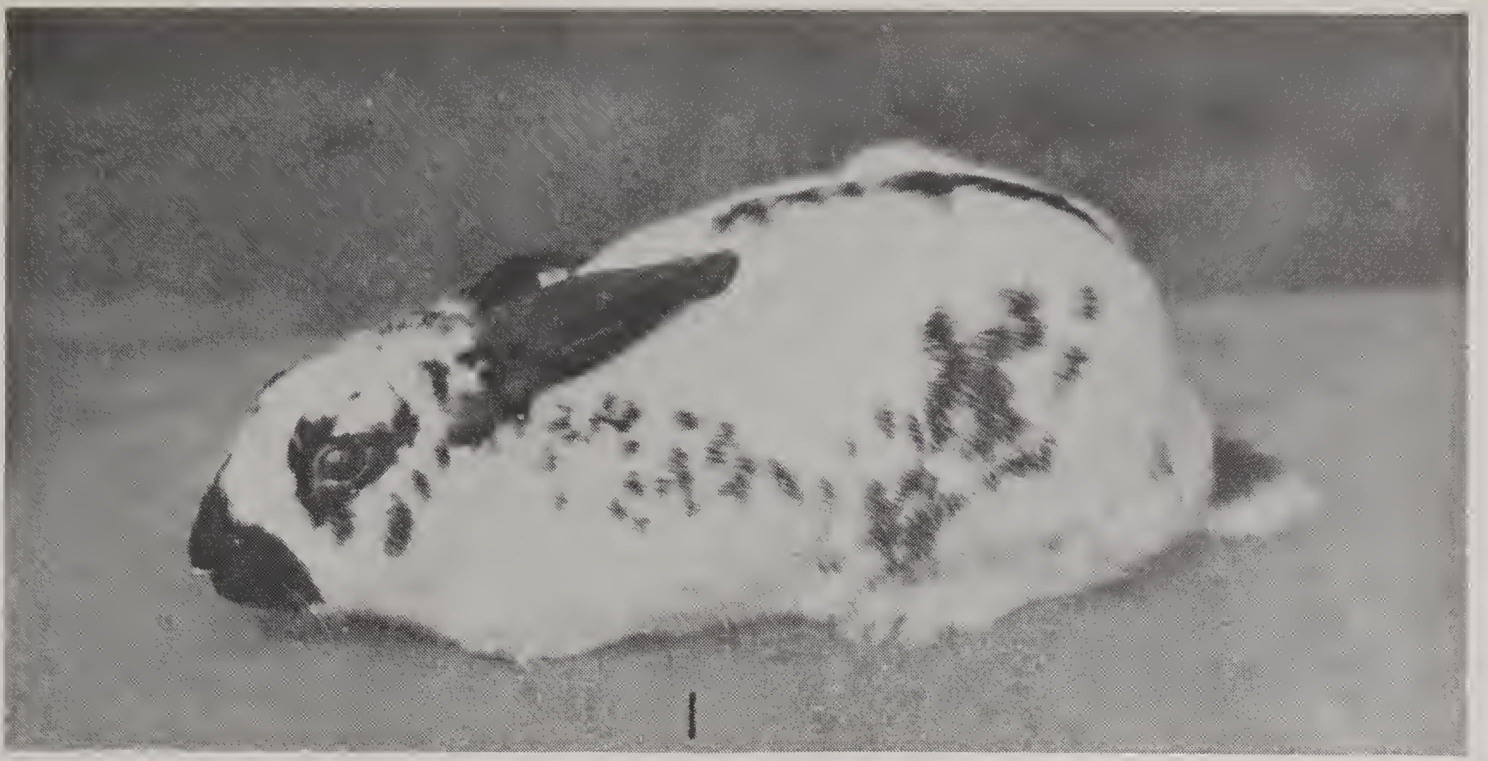

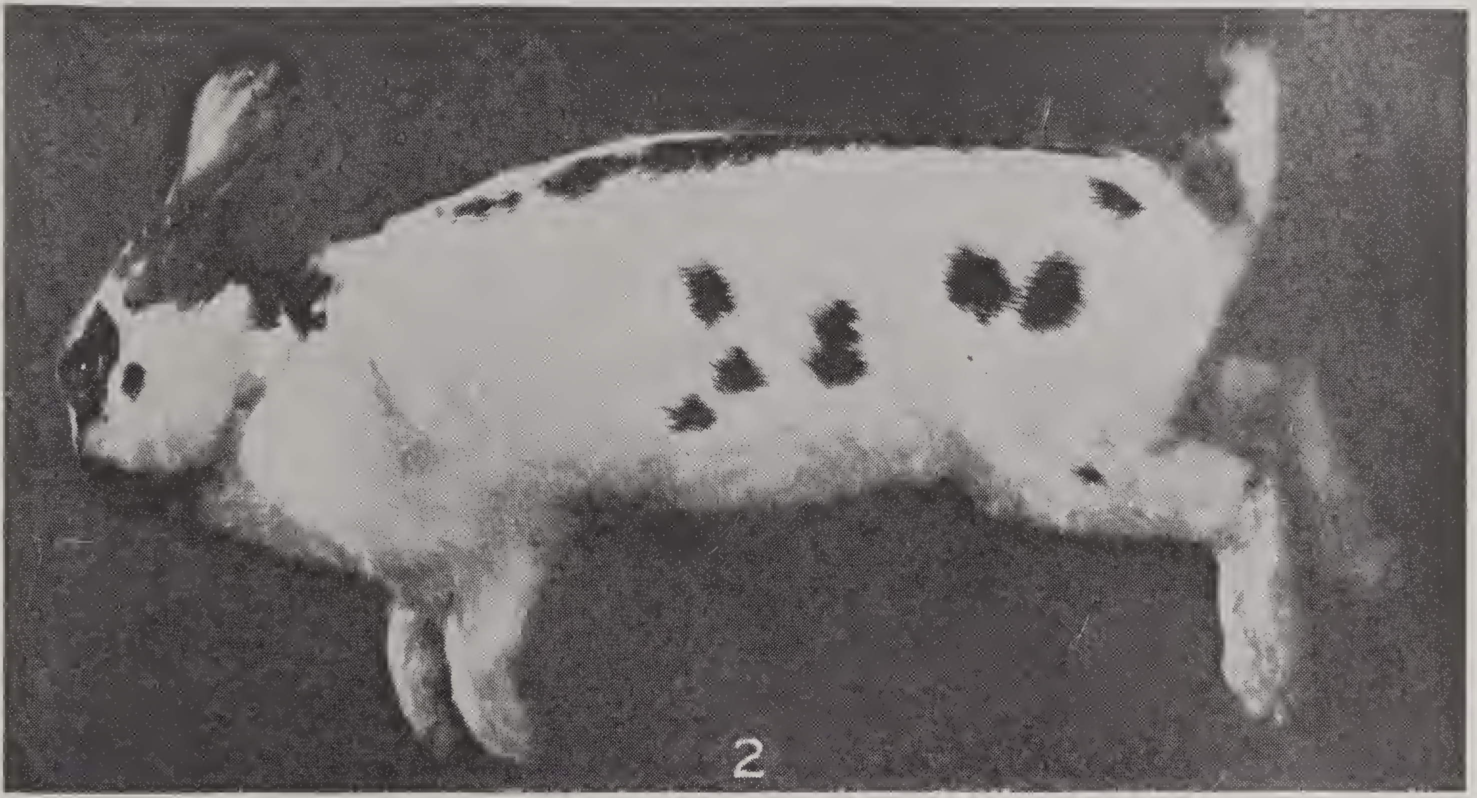

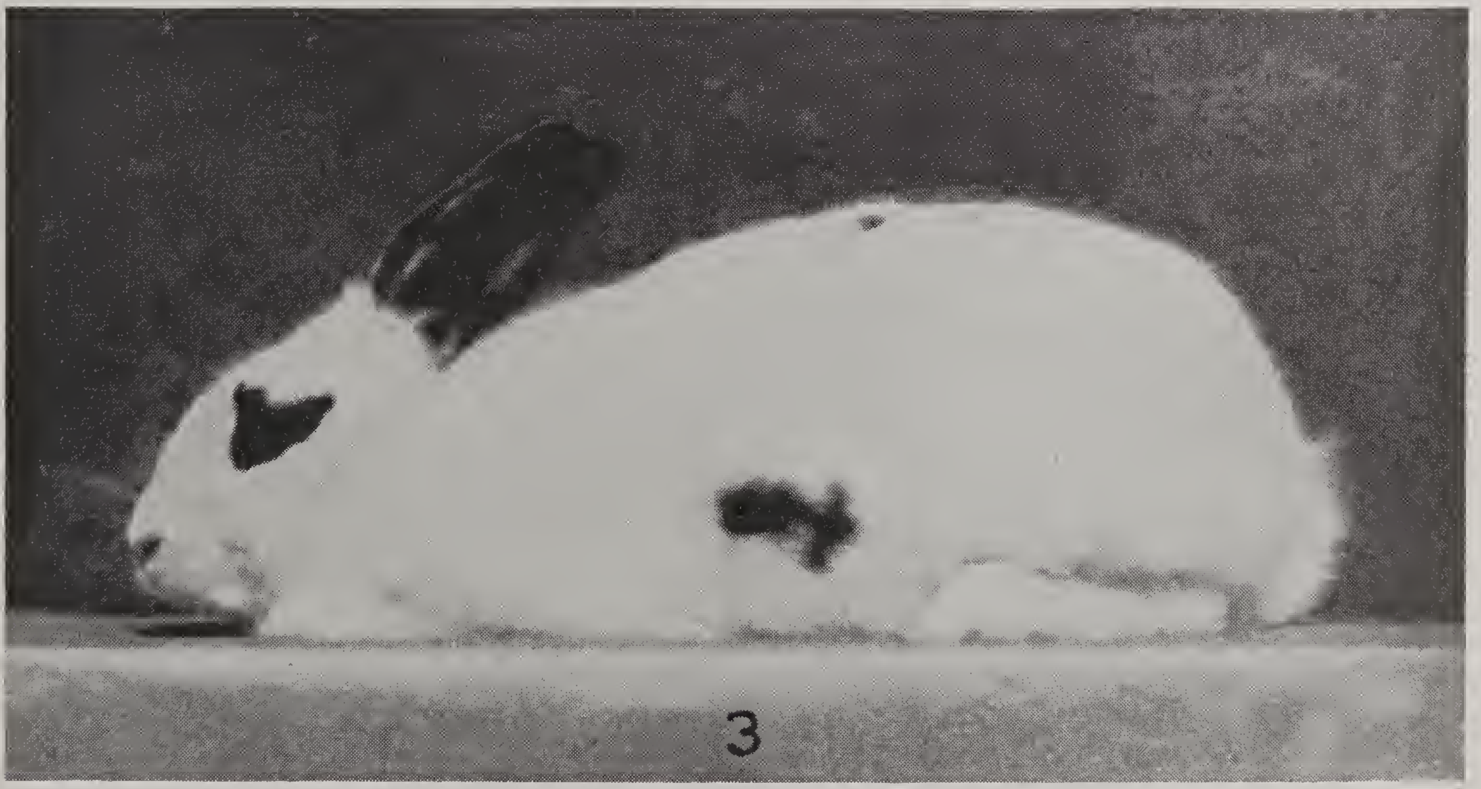

Fig. 1.—Heterozygous English rabbit, exhibition type, showing "butterfly" nose marking and chain of numerous side-spots desired by fanciers.

Fig. 2.—Heterozygous English of Checkered Giant variety, in which side-spots are larger and less numerous than in exhibition type (figure 1).

Fig. 3.—Heterozygous English rabbit produced by a cross of the Checkered Giant shown in figure 2 with a White-Dutch Angora female similar in pattern to Plate 5, figure 3. Note dominant short hair and greatly reduced English markings. Dutch marking is not in evidence either on rump or between eye and ear spots. Compare Plate 5, figure 3, and Plate 6.

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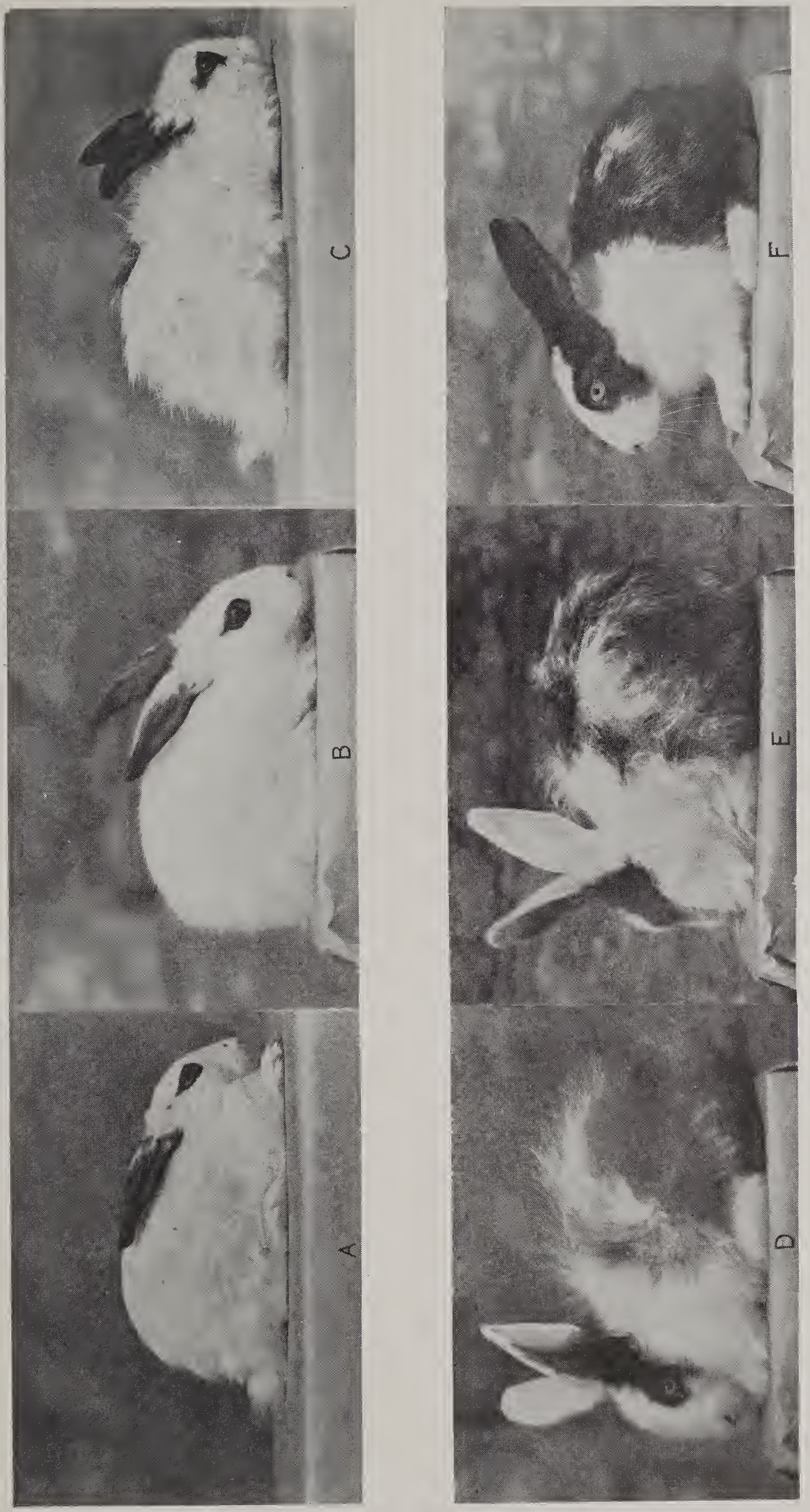

Group of six young produced by a back-cross of English male, shown in Plate 7, figure 3, with a Dutch-Angora female of grade 13. A and B, English young which are short-haired; D and E, Dutch young which are long-haired. These are the usual or non-cross-over classes. C, Long-haired English; and, D, short-haired Dutch; these are the exceptional classes and arise as cross-overs between pattern and hair-length.

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PART II

FERTILITY AND STERILITY IN THE NORWAY RAT, MUS NORVEGICUS

By Horace W. Feldman, Sc.D.,
Bussey Institution, Harvard University.

With five text-figures.

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FERTILITY AND STERILITY IN THE NORWAY RAT, MUS NORVEGICUS.

GENERAL INTRODUCTION.

The primary object of the present study was to investigate the occurrence and inheritance of sterility in mammals. The type of sterility which is due to gametic incompatibility is of especial interest. During the course of the work, a considerable amount of data was obtained which has to do with other phenomena of reproduction in mammals. The Norway rat, *Mus norvegicus*, was employed in the investigations because of its adaptation to laboratory use.

MATERIAL AND METHODS.

The rats used in this study consisted of three stocks: (1) 3 litters of the red-eyed yellow mutant variety (Castle and Wright, 1915), and their descendants in three succeeding generations from brother-sister matings. (2) 13 males and 14 females of the red-eyed yellow variety and their descendants in two succeeding generations from matings between individuals as distant in relationship as the stock would permit. (3) 11 males and 11 females from the fifty-second generation of Doctor Helen Dean King's strain of albinos which have been inbred by brother-sister matings.

These rats were kept in wire cages which had a floor space of 14 by 16 inches, and which provided a total space of about 1,400 cubic inches. Each cage was in a galvanized metal pan having sides 3.5 inches high. They were arranged on wooden racks in tiers. The floors of the pens were covered with sawdust, and paper waste was used for nesting. All cages were cleaned once a week. Not more than 5 rats were ever placed together in a cage. The ration which these rats received was essentially one of cereals supplemented with dried meat scraps and powdered skimmed milk. A high-protein dog-biscuit was before them at all times. Clean water was constantly available to all pens. About once each week a small amount of green food (grass, clover, lettuce or sprouted cereal) was provided. In general, the food and management were sufficiently uniform throughout the work to warrant their exclusion as variables in consideration of the data.

The plan of handling the rats was also very uniformly pursued throughout the experiments. All animals which were intended for observation in this study were weaned at the age of 30 days. At that time, they were marked, weighed and separated as to sex. Between the ages of 30 and 90 days, they were weighed at least once as a check on their growth. When 90 days of age, they were again weighed and mated for the first time. Each male to be studied was placed in a separate cage and the females which were to be his mates were added. At about the eighteenth day of pregnancy each female was removed to a separate pen. Within 24 hours following parturition she was placed with her new mate. No extended lactation periods were ever permitted. A mating which did not produce a litter within 44 days was considered provisionally negative and the female was placed with a new mate. To be efficacious, under the method of mating pursued in this study, examination must be made of the stock of rats in a thorough manner for the occurrence of sterility conditions. The mating of each male with each of his sisters, wherever possible, should produce recombinations of similar hereditary factors and therefore seems to be most suitable. This type of mating was used with the main stock and with the inbred albinos. The control stock was tested by mating distantly related individuals of each generation. Second-cousins were actually combined in most cases. These latter animals were maintained as controls on the inbred families, and are referred to as cross-bred in this paper.

The specialization of the methods with the view of analyzing the stock for inherited sterility has necessarily limited the value of the data on certain other points. Since the rats were all mated at the age of 90 days, no information was obtained upon the question of age of maturity. Likewise, the rats were discarded before they were a year old; information on senescence is therefore lacking. At the same time, the uniformity of the methods adds considerable value to the data on certain other points, such as promptness of mating, size of litter, stillbirths, growth, effects of inbreeding upon reproductive vigor, and the inheritance of these characters.

FERTILITY AND STERILITY.

The modes of reproduction exhibited by the higher plants and animals are highly specialized, and differentiated in the various groups. The gametophyte in the angiosperms is extremely sensitive and exacting as to conditions for the performance of its normal function. The same may be said of the corresponding stage in the vertebrates. Our present knowledge gained through research has revealed several conditions, both hereditary and environmental, which are capable of preventing the functioning of gametes within a species. Some degree of sterility is the result.

In normally hermaphroditic or monœcious forms, inherited self-sterility exists. Kölreuter (1764) first reported this condition in three plants of *Verbascum phœniceum*, although they set seed with pollen from four other species of *Verbascum*. Fritz Müller (1868, 1869) in comparative studies of self-sterility in *Eschscholtzia californica* associated the condition with excessive vegetative growth. Darwin (1875) discussed at length various cases known to him, and concluded that it was due to an excessive similarity of the gametes. He ascribed the fertility of other individuals in the population to an accumulation of small germinal differences between them. Jost (1907) working with *Corydalis cava* and Correns (1912) with *Cardamine pratensis* concluded that self-sterility was due to the retarded pollen-tube growth in the styles of the same plant. Compton (1913) in his work on *Reseda odorata* obtained evidence that self-sterility was inherited as a recessive mendelian character. East and Park (1917) and East (1919) interpreted self-sterility in *Nicotiana* on the basis of Mendelian unit factors.

East (1921), Anderson (1923) and East and Mangelsdorf (1925) have studied self-sterile groups within an inbred population from a cross between *Nicotiana forgetiana* and *N. alata.* The latter authors conclude that three allelomorphic, recessive, factors cause three self-sterile groups in this population. Correns and East report that slightly unsuitable conditions of growth may induce self-fertility in self-sterile plants.

Castle (1896) observed self-sterility in the ascidian, *Ciona intestinalis.* Morgan (1904, 1910, 1923) has concluded that the condition in this form is due to the inability of sperm to penetrate the test cells surrounding the majority of the eggs produced by the same individual.

A survey of the cases of self-sterility in angiosperms, which number about 100 in 35 different families, and of the case of *Ciona intestinalis* admits of several conclusions: Certain hereditary factors are capable of decreasing the fertilizing power of gametes which possess them. The factors produce sterility by preventing the union of gametes, and not by destroying the products of fertilization. The *modus operandi* is an inhibition of the haploid male element which possesses the factor, by the presence of the same factor in certain diploid maternal tissue with which this male element must have intimate contact prior to fertilization. The mechanism which produces the inhibition is not known.

Cases of inherited self-sterility and cross-sterility have not been reported in most of the systematic groups. The physiology of fertilization is not essentially different in the groups which possess cases from those in which it has not been reported. The possibility of inadequate tests is a consideration, especially in the unisexual or diœcious forms. In such forms, cross-fertilization is the rule, and, as a result, unlike genetic complexes usually meet. Our present knowledge indicates that frequent unions of like genetic complexes are necessary to demonstrate the presence of these sterility factors.

There is no good reason apparent for the non-existence of inherited cross-sterility in such a group as the mammals; yet there is very good reason for a lack of its demonstration. Mammals, in general, are capable of extended migrations in their search for food and more suitable environments. In most localities, a sample of even the common species would be found to contain at least some extrageneous admixtures. Coupled with unsuitable matings are the scanty observations on all except our domesticated forms; and even in these the absence of suitable tests. An examination of at least one species of mammal for the occurrence of hereditary cross-sterility seems justified.

Of the available species, the Norway rat, *Mus norvegicus,* is the most suitable for such an examination. The various domestic varieties of the rodent are most prolific under laboratory conditions. They are relatively free from disease and at the same time are more uniform under our methods of management than are other forms. Added to these advantages are the relative ease and small expense with which a colony of rats can be maintained in good condition.

Knowledge of self-sterility and cross-sterility, or gametic incompatibility in the species noted above, compels us to resort to inbreeding with the rat. Brother-sister matings are the most efficient method of producing a homozygous condition in a population of unisexual organisms. Moreover, the self-sterility already recorded exists in hermaphroditic forms. In order to imitate that situation as closely as possible, the rats were tested by matings between litter mates, and each generation was reared from such matings. A smaller stock was reared in parallel generations by crossing various animals as widely as possible. This was done to combine genetic complexes which were similar yet not as homogeneous as those produced by brother-sister matings. These cross-bred rats were also tested by wide combinations.

The systematic mating of each male with each of his sisters in the 3 parental or first generation litters, A, B, and C, yielded no sterile matings. The parental matings of the cross-bred stock also yielded 100 per cent fertility, but there were only 8 matings in this case. This small group is not included in Table 15 which contains the other data on fertility.

The second and third generations of brother-sister matings produced 85.1 and 85.4 per cent respectively, of fertile combinations. The fourth generation was lower with only 66.3 per cent of fertile matings. This generation showed a low state of vigor which partially explains the low fertility. The three generations of cross-bred rats showed higher percentages of fertile matings ranging from 87.2 to 90.5 per cent. The differences between the inbred and the cross-bred stocks in percentage of fertile matings do not indicate a more frequent occurrence of cross-sterility in the former. The explanation is to be found in part, at least, in the greater vigor of the cross-bred stock.

The writer was fortunate in having a number of rats from Doctor Helen Dean King's closely inbred albino strain. The animals, which were tested by brother-sister matings, composed 5 small litters produced by 52 generations of brother-sister matings. The low figure of 75 per cent of fertile matings in this vigorous strain is very interesting, for its history is very favorable for the appearance and demonstration of sterility factors. The intense inbreeding to which these rats have been subjected has probably reduced them to a practically homozygous condition. Theoretically, brother-sister matings produce homozygosity for an infinite number of factors in 99.64 per cent of the population in the twenty-sixth generation, provided there is no genetic linkage.

Table 15 gives the distribution of the sterility cases in various subgroups of the population, with a general classification based on observations of the animals concerned. The majority of the sterile matings could be explained in part by totally sterile animals, and in part by sterility conditions acquired at various stages of the rats' lives. The small percentage of sterile matings which could not be definitely ascribed to the condition of either partner are of especial interest. In them must be included cases of cross-sterility or gametic incompatibility, if any such occurred. The distribution of the undetermined sterility in the population did not indicate an underlying cause which was inherited. At this time it seems more reasonable to ascribe it to temporary or permanent sterility conditions (of the females in most cases), which were not diagnosed by the methods employed. The unusually high frequency of 10 per cent in the matings of the King strain of albinos is perhaps worthy of note. Larger numbers of rats of this strain are now being tested.

The cases of sterility of determined causality fall into two broad groups. One group was due to a total or congenital sterility of one or both of the partners. The other group was due to sterility conditions acquired during the life of one or both partners. In the former, the animals failed to produce offspring in any mating. In the latter, some of the matings involving any particular rat resulted in offspring, but at some point in its life conditions of health interfered, so that the following trials were negative.

Nine individuals or 2.4 per cent of the males and 25 or 5.4 per cent of the females proved totally sterile. Most of these sterile rats were of generally low vigor, weak biotypes. The weak types were restricted to the inbred stock. Brother-sister matings were undoubtedly responsible for the low vigor. Four totally sterile females attained the body-weight of their brothers and exhibited other masculine characters in their coarseness and pugnacity. One of these showed encysted ovaries to a very marked degree, so much so, that little ovarian tissue remained. Another had a decidedly atrophic uterus. Several sterile rats of both sexes failed to show abnormalities of any sort.

The great majority of the instances of acquired sterility were accompanied by the symptoms of rat pneumonia described by Jones (1922). The pneumonia was especially prevalent in rats of subnormal body-weight, and on the average became aggravated in such animals at a much earlier age than in the larger ones. This disease and a few cases of dysentery were the only causes of acquired sterility determined in the males. In addition to these, females became sterile from causes incident to former pregnancies. Prolapse of the uterus was especially frequent in the first two generations of family C, one of the three groups of inbred rats. Infection of the uterus from retained products of former pregnancies were not infrequent. Encysted ovaries also caused the cessation of fertility in a few females. Tumors upon various tissues, either directly or indirectly brought on sterility in at least ten females.

Entirely unfruitful matings are the only absolute criterion of the occurrence of inherited sterility, of the nature of gametic incompatibility. But a heterozygous condition of a male for such sterility could exist. In that case, part of his sperm only would carry the factor, and, as a consequence, they alone would have impaired fertilizing power. The large number of sperm which a rat ejaculates in coitus would tend to obscure a situation of that sort. At most, we could expect to observe it in a reduced litter. This would be extremely difficult to do because of the extreme variability which normally occurs in the size of litters in the rat. In the present work no evidence was secured to indicate that any part of this variability was due to incompatibilities.

Fertility, when defined as fruitfulness of a mating between a given male and a given female in terms of living offspring, is actually a composite of a series of phenomena. These in turn are dependent upon factors of heredity and environment. Frequency and size of litters in the rats appear to be practically an expression of the vigor of the individuals concerned.

Further, they are in most cases an expression of the vigor of the female. The male rat, unless he is sterile, inseminates with a quantity and quality of secretions which are capable of producing a full-sized litter. It is necessary, therefore, in order to analyze fertility, to study these factors from as many aspects as possible.

REPRODUCTIVE VIGOR AND GROWTH.

ŒSTROUS CYCLE AND PROMPTNESS OF MATING.

The number of litters which a female bears in a given length of time is a direct indication of one phase of her reproductive vigor. The determination of the length of time from the date she is placed with a fertile male until she bears a litter is a good measure of this factor. The gestation periods fall between 21½ and 22 days. The number of days in excess of 22 which elapse before parturition is, therefore, the time which the female spends with the male before successful mating is effected.

In these studies the dates of mating and of parturition were recorded. Lactation did not occur in any of the females, for their litters were destroyed within 24 hours following parturition. Its effect in extending the gestation period, as described by King (1913), was therefore eliminated. It was found that the range of the time periods between mating and parturition was from 22 to 44 days. The former period represented a successful mating on the first day the rats were placed together. The latter represented a lapse of 22 days before pregnancy resulted. At the expiration of this maximum period the mating was broken and considered provisionally negative. Certain litters were born after the female was placed with the new mate, however. In analyzing the distribution of the matings as to the promptness of conception, first litters have been separated from subsequent ones. Figure 1 gives the frequency distribution.

Of the 385 first litters, 315, or 81.8 per cent, were born within 29 days after the female was mated. Of the 1,261 later litters, 927, or 73.5 per cent, were born within that period. These litters may be considered prompt and do not, in all probability, indicate the presence of delaying factors. The cause of the difference between the percentage of prompt matings among first litters as compared to those which followed is not clear. The increased susceptibility of the older animals to slightly inadequate hygiene may explain it.

The studies of Long and Evans (1922) on the œstrous cycle in rats determined that its average length in 92 per cent of the instances was 4.8 days. The minimum was 3 days and the maximum was over 13 days. The average of the total of 1,999 instances was 5.4 days. Those authors note that various factors of hygiene tend to extend the length of the cycle. They mention diet (undernutrition), regularity of feeding, frequent handling, crowding, copulation and lactation. They conclude that the normal cycle is 4 days, and that the high frequency of longer periods is "due to slight, but constantly operating, inadequacies in the hygiene."

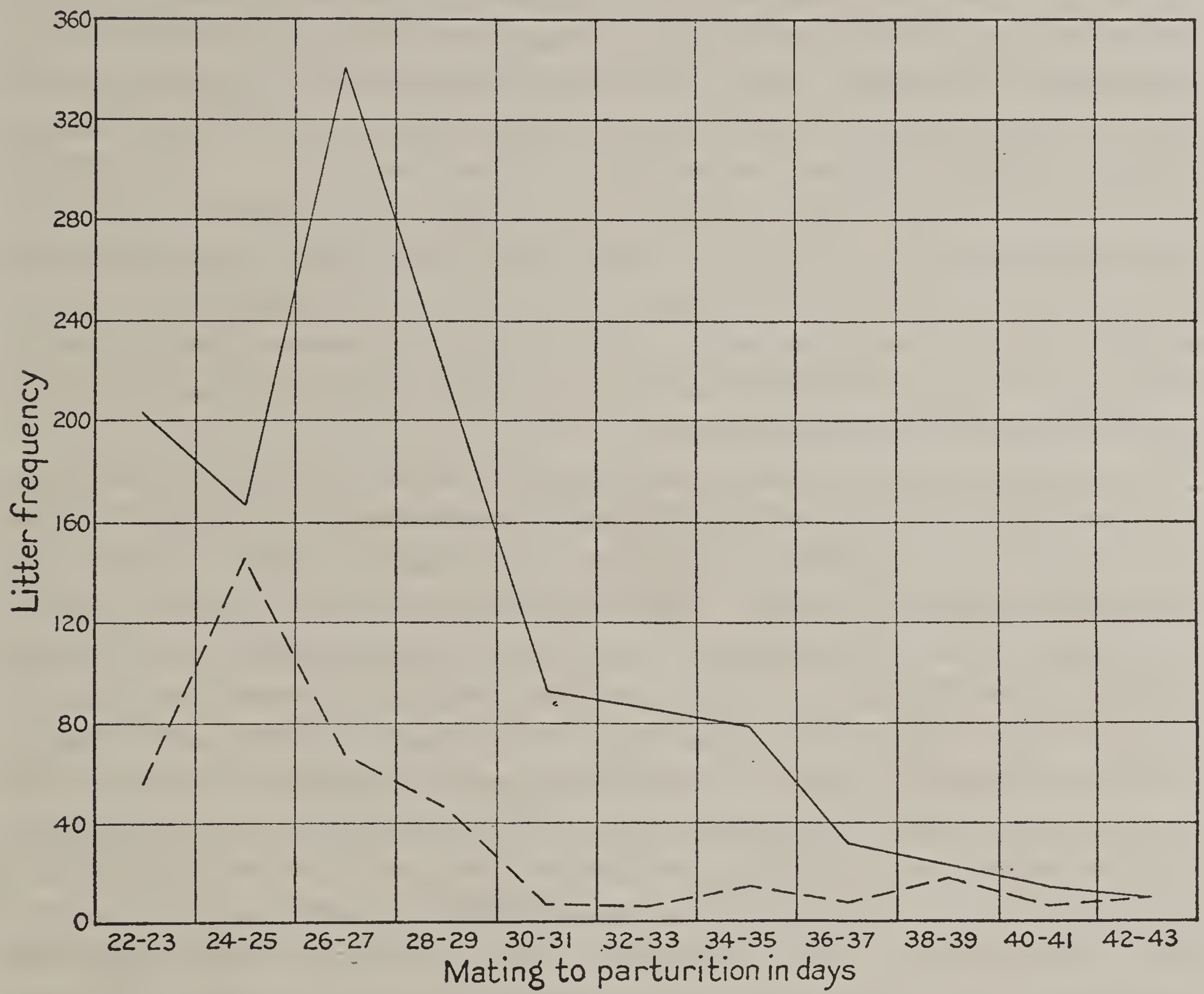

FIG. 1.—Variation in the periods between mating and parturition. Broken line represents first litters. Solid line represents second and subsequent litters.

With a knowledge of facts concerning the œstrous cycle, the interpretation of mating results is facilitated. The matings which were successful in less than 29 days may be assumed to have resulted from the first œstrum and accompanying ovulation after the female was mated. Those from among the second, and subsequent pregnancies which occurred on the twenty-second day, probably resulted from the ovulation which immediately followed the preceding parturition. This explains their relatively high frequency. The distribution among first matings is what may be expected if conception occurred at the first ovulation on the basis of an average cycle of 5 days. The females were placed with the males in a random fashion in regard to the stage of the cycle they were in. In the second and subsequent matings, the distribution indicates that, in most cases, pregnancy resulted in a like manner from the first ovulation following mating. But in these instances most of the rats were in the third or diœstrum stage of the œstrous cycle when mated. The exceptions are those, as noted above, which were in the second or œstrum stage.

In both the first and subsequent matings, a certain percentage was delayed. Table 4 gives the age distribution of such matings. It may be noted that the first age group shows a low percentage. This is of no significance because this first age group includes only first matings which were successful within 30 days after they were made. Only those which were delayed 4 days or less fell in this group. The various age groups show no significant deviations. The matings which involved females older than 270 days showed, perhaps, a slightly higher frequency of delayed instances. It is sufficient to note that 56.4 per cent of all matings required more than 26 days before conception resulted. In other words, in the rats studied, more than half of the matings did not result from a first œstrum of normal length after the female was placed with the male. That the delays were primarily due to a delayed œstrum is the belief of the writer. This, in turn, was founded on the subnormal condition of the females.

The age fluctuations of the time required before the birth of the litter, after the female was placed with her mate, are presented in Table 3. Here, again, there are no significant variations, although the three advanced age groups show higher averages. The general average was 29.0 days, which means that about 7 days elapsed before the average mating was effective. The rats over 270 days of age were less prompt in conceiving than the younger ones. This result, with the result shown by the percentage of delayed matings in Table 4, indicates a more irregular function of the reproductive system in advanced age. This evidence is less conclusive, however, when we know that the general vigor of the individual rat is reduced with advanced age. The susceptibility to adversities of environment and hygiene is thus increased. The conclusion seems warranted then, that advanced age affects the regularity of the reproductive function indirectly through its effects upon the general vigor of the individual. The decrease of fecundity in women described by Duncan (1876) and others is comparable. The expectation of life in humans when compared with the fecundity denotes a strong causal relationship at the various ages.

The promptness of mating as an individual characteristic was not a marked one. Very little constant variation was found among individual rats. The population included in this study when considered from the standpoint of the average length of time for the various matings of the individual showed a standard deviation of 3.96 days. This was due, however, to chance occurrences of delays of various extents in one or more of the matings involving each individual. Promptness of mating of the individual rats showed a slight causal relationship to the individual's vigor. When the average length of mating time was correlated with the body-weight at 90 days, a coefficient of correlation of — 0.193 ± 0.033 was obtained (Table 5). This may mean that the larger females tended to mate more promptly.

SIZE OF LITTER.

The size of the litter or the number of live young produced at any parturition is the best index of the female rat's reproductive vigor. At the same time, it is a very inadequate index of her inherited fecundity. This is primarily due to the number of factors, principally of hygiene, which tend to detract from the normal or maximum litter size.

The female rat is capable of giving birth to at least 14 young at one parturition. King (1916) has reported the maximum of 16 young in one litter. The reasons for the deviation from this maximum by the average size are partly explained by the resorption of embryos. Long and Evans, *loc. cit.*, found that in 50 cases the average number of ova in the oviducts was 9.6. The same females which gave the ova count produced litters averaging 6.4 young. Thus almost one-third of the ova in the oviducts failed to produce young. The reasons, *a priori*, for the difference between ova count and the number of young have not been determined. The failure of ova to become implanted and the death and resorption of embryos are the possible events to bring this about. The exciting cause of resorption of embryos is probably the derangements in uterine circulation produced by an inadequate blood supply. That a significant proportion of fœtuses die too late in gestation to be resorbed is shown by their remains in the products of parturition.

Whether the particular rats which were studied in this work were subject to genetic variations of the number of ova produced at each ovulation was not determined. The gross fecundity was unknown. The number of live young in each of the various litters was variable. The average of the 1,740 litters recorded was 5.80. Other investigators have found that the average litter is somewhat higher: Crampe (1884) found 6.3; King and Stotsenburg (1915) found 7.0; Long and Evans, as reported above, found 6.9; and King (1924), who included the entire litter series of 148 females, found 6.1.

The most constantly operating factor in determining the size of litter was the age of female. King (1916) has recorded the breeding history of 76 vigorous F_2 females from a cross of Norway × albino. The litter size increased with age until the female was 7 months old; and after the age of one year a sharp decline occurred. Age of female and not the position of the litter in her breeding history affected litter size. In the present work the effects of the females' ages upon the average size of litter may be observed from Table 6. Females 90 to 120 days old produced an average litter of 5.60 young. The age groups of 120 to 150 days and 150 to 180 days produced averages of 6.13 and 6.27 young respectively. The latter marked the maximum litter size. The females at more advanced ages produced averages which fluctuated between 5.85 and 4.97 young.

The effects of age upon litter size are, in common with the effects upon other characters, dependent upon the individual's vigor. Beyond the age of about 180 days, increased age is accompanied by a steady decrease in vigor and in resistance to adversities of the environment and hygiene. In the case of litter size, the averages of Table 6 do not fully portray these effects because the population did not remain constant. A progressive elimination of the weaker individuals tended to maintain a higher average than would have otherwise existed.

In an effort to determine the general relation of vigor to net fecundity or litter size, the correlation was determined between body-size at 90 days and average size of litter (Table 9). The weight at 90 days was taken to represent best the individual female's vigor of growth at a time when size was unaltered by reproductive activity or the unreliable increments of advanced age. The average size of all the litters produced by an individual was thought to be a better measure of her fecundity than the size of her first litter or any other single litter. This procedure tended to eliminate minor fluctuations of the individual litters. A correlation coefficient of $+ 0.356 \pm 0.030$, signifies a small, yet significant causal relation between weight of female and size of her litters. Females of vigorous growth tended to bear more young than those of less rapid development.

Other possible factors affecting litter size are the health and condition of the female. Using the body-weight at parturition as an index, it was hoped that some light might be cast upon this point. The females comprising the fourth inbred generation, the third cross-bred generation, and the fifty-second generation of King inbred albinos were weighed as soon after parturition as it was certain that all the products had been expelled. This proved to be within 24 hours after the first fœtus was born. Variation in size of the litter probably introduced some error. We know that in swine, for instance, sows will often contribute from their own body-substance for the prenatal as well as the postnatal growth of a large litter.

The data on weights are recorded in Table 7; the variation in size of litter produced by the 81 females whose weights are tabulated are also shown in this table. Since age affected body-weight and litter-size alike, the various age groups have been tabulated separately. A natural increase in weight was to be noted in these females during reproductive activity. The age group of 90 to 120 days averaged 165 grams; the increase was steady to the age group of 240 to 270 days, which averaged 254 grams. This increase of weight with age, as indicated by the averages, was probably accentuated, however, by the elimination of the weaker individuals in the advanced age groups. The average weight of the females producing the various sizes of litters showed that in general the larger litters were produced by the heavier females. An inspection of individual cases revealed no general relation between weight at parturition and number of young in the litter produced. The short breeding history of the females increased the difficulty of observing such a relation.

The frequency of litters denoted relatively little variability in the size of litters produced at early ages. The variability proceeded to increase with age. The small samples made a determination of the mode for each age group of little value. The average litter of all ages was 5.74 rats; the modal class was 5 rats per litter.

The various environmental or external agencies which were suspected of influencing the size of the litter in these rats were difficult of analysis. Seasonal variations of temperature and humidity were an exception, however. The average size of the litters born during each month of the year was determined from April 1, 1923, until June 1, 1925. The fluctuations proved to be slight, yet similar, in corresponding months of each year. The results for each month are combined in Table 8. June seemed to be the most favorable combination of seasonal conditions; in this month the maximum average litter of 7.3 young was born. The preceding months were fairly uniform with averages which fluctuated between 6.5 and 6.3 young per litter. June was followed by a rapid decrease until the low average of 5.2 in October was reached. During November and December the litter average increased.

The temperature of the current month does not seem to have been the chief factor in the seasonal fluctuations of the size of the average litter. The months of May and October and of June and September have very similar temperatures in the vicinity of Boston, yet the average litter in the spring months is appreciably higher than in the fall. Upon the basis of temperature, the most plausible explanation lies in assuming a devitalizing effect of the high temperatures (and high humidity) of July and August which did not fully manifest itself until October. King and Stotsenburg, *loc. cit.*, failed to observe seasonal variation in the size of litter in their rats. Wright (1922) observed in guinea-pigs that the size of litter as well as other characters of vigor was lowest in the winter and early spring, and reached a high level in the summer and fall. This variation was ascribed to a fluctuating supply of suitable green food. In the rats of the present study the food was uniform and was therefore of doubtful importance as a cause of the variations in litter size.

The variations of litter frequency—litters per mated female—seem to have been affected by the season also. Monthly averages show a high general frequency in the months preceding August. In June the highest frequency of 0.96 litters per female occurred. In November the frequency reached the lowest point of 0.19 litters per female. The month of April was exceptionally low, however. Litter frequency seems to have been more sensitive, on the whole, to irregularities and deviations from the optimum temperature than litter size. The reactions of the two characters were similar though to a given type of change. The general trend of reproductive activity in these rats may have been influenced by the ancestral yearly cycle, in addition to temperature. Although the food supply was constant in the laboratory, the persistence of a racial trait based on a variable seasonal supply is possible. The relatively high frequency and large size of litters born in the spring and early summer corresponds to the behavior of the Norway rat and similar rodents in the wild state.

BIRTH MORTALITY.

In the preceding discussion of size of litter in rats, stillbirths were mentioned as a factor which detracts from the net fecundity. The death of fœtuses either *in utero* or at parturition was found to account for a considerable part of the difference between the number of ova liberated at an ovulation and the number of live young born from the resulted pregnancy. The true frequency of dead young was somewhat decreased by the habit of some females of devouring the lifeless individuals with the placenta.

Stillbirths had their earliest origin at the stage of fœtal development when resorption is no longer possible. From that point they originated at various stages of prenatal growth and during parturition. A certain proportion was caused by a severing of the umbilicus too close to the body by the mother. Such individuals were distinguished by their pale appearance in addition to the condition of the umbilicus. The underlying cause of stillborn rats was in some cases an inherent weakness of the fœtus. In the majority of the cases, however, the weakness of the female was the fundamental factor. The original product of conception over-taxed the reproductive powers. Stillbirths were the result of the last readjustments before parturition, or the result of delayed birth due to a weak uterine function at parturition.

The percentage of stillbirths in a total of 31,670 rats observed by King (1921) was 1.3 or 415 individuals. The causes of birth mortality included malnutrition, size of litter, suckling of young, age and condition of the female. King observed a higher frequency of stillbirths in the fall than in the spring.

In the present study a total of 875 stillborn young rats and a total of 10,104 live rats were recorded. The stillbirths thus composed 7.9 per cent of the total products of parturition. This is considerably higher than the percentage reported by King. The rats in this study were perhaps weaker than those with which King dealt.

The frequency of stillbirths did not fluctuate significantly with the age of the female; although, from Table 10, it may be seen that the percentage was lowest at the ages of 120 to 150 days. This was the age period of greatest reproductive vigor of the females. The litters which contained one or more stillborn young have also been tabulated according to the age of the female at the time the litters were born. Table 11 shows that the frequency of litters containing stillborn young is not markedly affected by the age of the female. However, the percentage is lowest at the ages of 120 to 180 days. The frequency of stillbirths among litters, as well as among individuals, was lowest when the females were in the most vigorous condition. The proportion of dead young in litters of various sizes is of interest in relation to the vigor of the female. A small percentage of stillbirths occurred in litters of all sizes. High percentages occurred in all of the small litters produced by weak females and in the extremely large litters of vigorous ones whose condition became impaired a few days before parturition. We have good evidence therefore, that stillbirths were due in part, at least, to impaired vigor of the female.

GROWTH.

The study of growth has been of only secondary importance in this work. Growth and especially body-weight at certain ages have been used as an index of the general vigor of the subgroups of the population and of the individual. The data disclose several facts regarding the phenomenon in these rats.

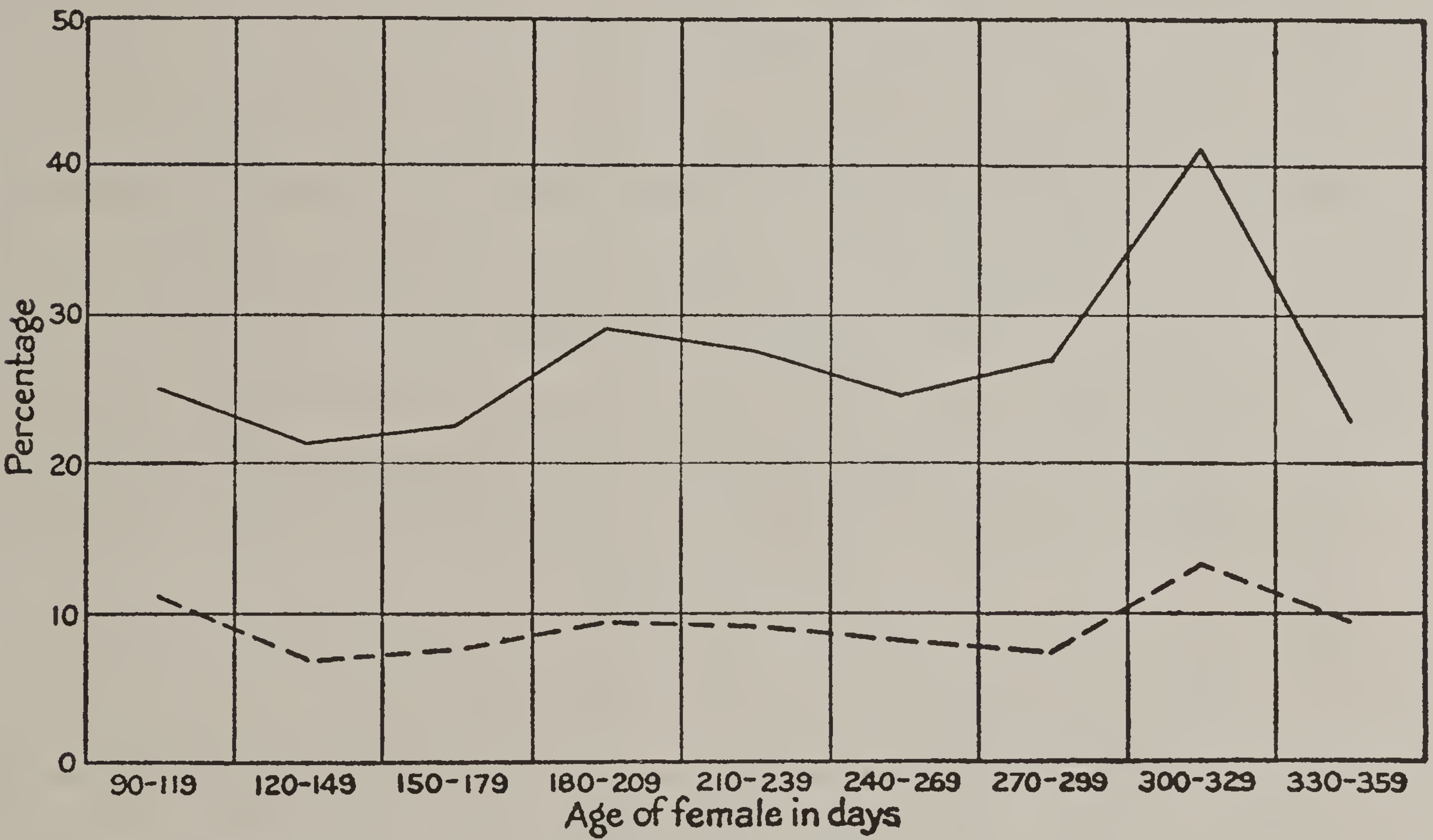

Fig. 2.—Birth mortality and age of female. Broken line represents frequency of stillbirths in percentage of live young. Solid line represents frequency of litters containing one or more stillbirths in percentage of total litters.

Growth in rats is a coordinated increase in size which is subject to various influences from within the individual, from the race through inheritance, and from the environment. The differences of size among rats, as among other organisms composing a homogeneous population, is probably due to differences in cell numbers rather than to differences in cell size. Superficial examination indicates that the increased cell proliferation of the various organs is proportional to their relative size. Characters affected by the sex hormones comprise an exception, however; for after sexual maturity some organs and especially some skeletal characters tend to lose identical size relations in the two sexes. Most of the organs of the rat show sex dimorphism.

The growth of the rats involved in this study corresponded in some particulars to the growth of albinos described by Donaldson (1924), and by

King (1915). Males, on the average, were heavier than females at all ages. Those authors observed an average weight for males of 306 grams at the age of 365 days and for females of 223.1 grams at the same age. Their rats continued to increase in weight to that age.

The rats of this study were on the average smaller at all ages than those studied by the above authors. From Tables 12 and 13 and figure 3, it may be noted that the increase in weight was greatest at the early ages; after the age of 180 days it was small. The fluctuations beyond this age were due to the variations in the composition of the population; the weaker, smaller individuals were eliminated by disease, while many of the more vigorous ones were discarded because the desired fertility tests on them had been completed. The maximum average of males occurred at the age of 300 days, and of females 30 days later. The trend was upward in both sexes at all ages, however.

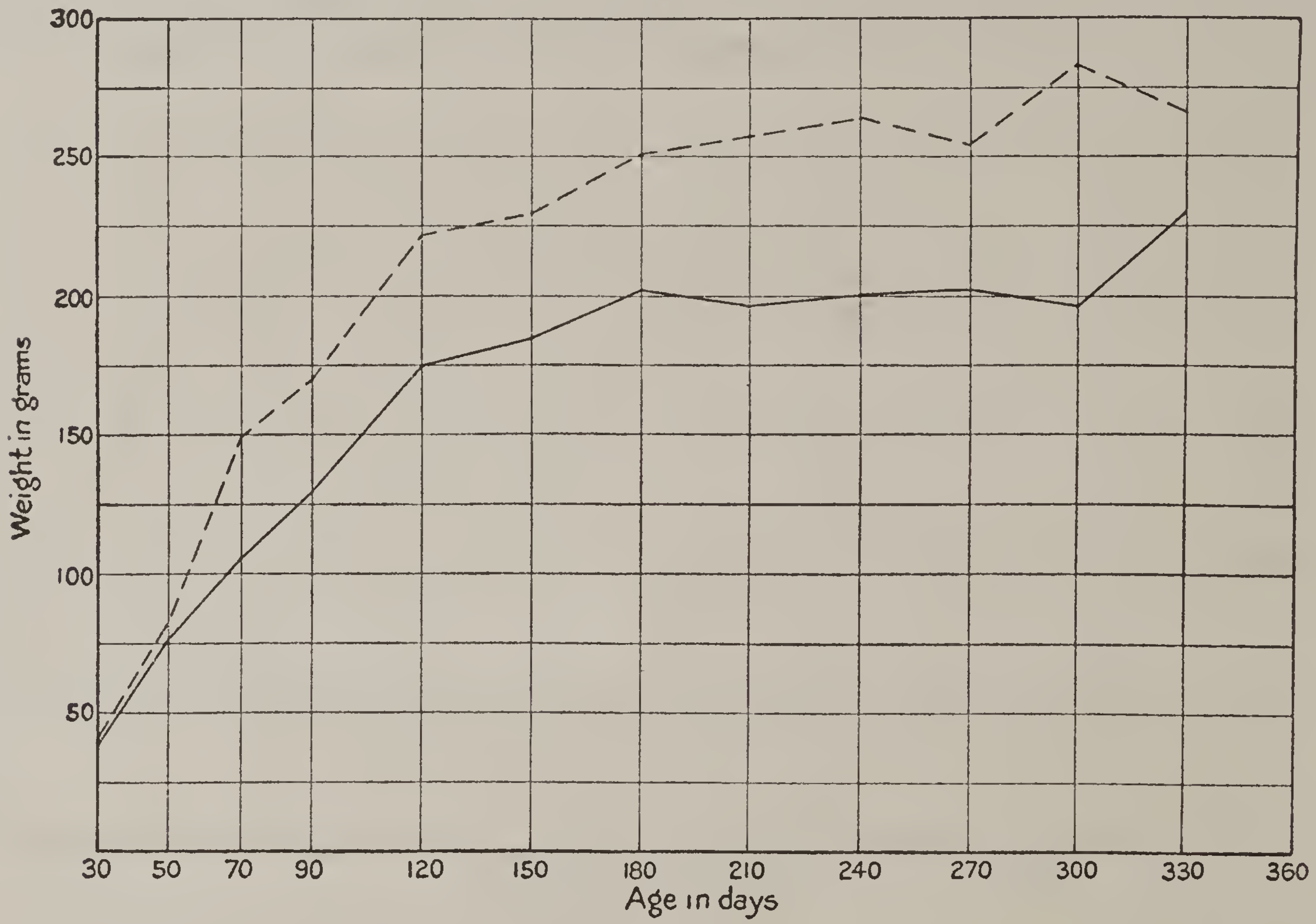

Fig. 3.—Growth curves of male and female rats. Broken line represents growth of males. Solid line represents growth of females.

Males tended to be heavier at all ages than females. The growth was practically parallel at most ages. The data show that the difference of 45 grams in weight was gained by the male almost entirely between the ages of 50 and 70 days. During that period of sexual maturing the average male gained 67.5 grams while the average female gained only 29.9 grams. The growth of the female was a uniform gain of 1.5 grams per day between the ages of 30 and 120 days. The growth of the male, on the other hand, was not uniform. The rapid gain between the ages of 50 and 70 days was followed by a more retarded growth during the period following.

Growth in the rat appeared to be extremely variable. In addition to the inherited determiners of body-size, a large series of other factors produced effects which have somewhat intricate interactions. The environment, food, cage conditions and other factors of hygiene all tended to detract from the attainment of maximum size. Rats at the age of 120 days for example, which were kept in groups of 5 to the cage, average as much as 25 grams less in weight than litter-mates which were kept in groups of 3 in a cage. Litter size was also a factor. Members of large litters tended to be smaller than those of smaller litters. The size differences at birth in this case were increased by deficient nourishment received during the first 21 days of post-natal life in the case of members of large litters.

The factors which detract from maximum growth show a differential action in the two sexes of rats. The female is much more uniformly affected than the male as shown by the variability of the weights at various ages. Table 14 contains the distribution of the weights at the ages of 30, 50, 70 and 90 days of some of the rats (first, second and third inbred generations and second and third cross-bred generations). The variability of the male was at least equal to that of the female at each of these ages. From the data it would seem that the female became more uniform with advanced age. At the age of 70 days the male showed a marked tendency to vary. The latter age marked sexual maturity and there is some indication that that stage of development is less stable than other stages and in the male is less stable than the corresponding state of development in the female.

INHERITANCE.

EFFECTS OF INBREEDING UPON REPRODUCTIVE VIGOR AND GROWTH.

A decidedly adverse opinion has been generally held regarding the effects of the mating of close relatives upon the offspring. The majority hold even yet that such matings inevitably lead to a general deterioration of the stock. Not only is the practice of animal and plant husbandmen governed by this, but the marriage laws of most states prohibit consanguineous unions. The practical results obtained in most of the higher animals provide grounds for this belief. Among plants and lower animals the situation is not as constant, for certain species seem well adapted to combinations of very similar germplasms. Hermaphroditic forms are the most striking example.

Experimental evidence in both animals and plants admits of certain generalizations. In those species which normally reproduce by the crossing of somewhat distantly related individuals, the effects of inbreeding vary in rate and extent. The deterioration is essentially a loss of vigor manifested by retarded growth and weakened constitution. Degeneration does not accompany inbreeding in most cases and can not be considered one of its results. The results, as first interpreted by Shull (1908) on the basis of Mendelian inheritance, indicate that repeated recombinations of similar germplasms cause a segregation of biotypes. In order to fit an interpretation to the observed facts, it may be assumed that the hereditary factors which produce vigor in the individual have recessive allelomorphs. Crossbreeding maintains a preponderance of the dominant factors in most of the individuals. Upon this Mendelian explanation an asymmetrical or skewed variation curve would be expected in the generations following inbreeding. A symmetrical distribution is actually observed in most cases, however. Jones (1917) explains these results by assuming that the dominant factors are linked, which reduces the number of recombinations.

The experiments of King (1918, 1919) on rats and Wright (1922) on guinea-pigs have contributed considerably to a knowledge of the results of inbreeding in mammals. These two experiments agree in the results obtained in general. The brother-sister matings tended to isolate various types which showed less variability as the work progressed. A general deterioration in characters of vigor and fecundity was especially noticeable in the early generations of the inbred animals. Lines became identified in the guinea-pigs for varying degrees of vigor in each character. Except in cases where characters interacted upon each other, for example the tendency of large litters to decrease individual birth-weight, no association between the various characters appeared. In the rats, selection of superior individuals produced a strain which excelled the general stock in growth and fecundity.

The present studies have progressed sufficiently to produce evidence upon early results of continued brother-sister matings with the particular rats which were used. The tables which include the data on promptness of mating, delayed matings, size of litter, stillbirths, growth and sterility, show the behavior of these characters in each of the inbred generations. The data on the cross-bred rats and those of the King inbred stock are also included in these tables. Table 19 gives the comparative ranking of these subgroups of the population in certain of the characters. In Figure 4 curves are plotted which show the behavior of these characters from generation to generation of inbred animals.

In a discussion of the results it seems advisable to consider, in addition to the data, other factors which may have influenced them. It is to be noted first that the three original, foundation litters, A, B, and C, of the inbred group were composed of exceptionally vigorous rats, as evidenced by their body-weight and reproductive history. They were on a par with the cross-breds and the highly selected King stock. It is to be expected that their progeny would show a regression toward the average of the population. The second inbred generation was composed of practically all of the young produced by the three litters, and was an average sample of rats in the writer's estimation. They were inferior to their parents in all of the characters of reproductive vigor and growth, except percentage of stillborn offspring. The differences are not significant in any character, however, for the parental generation could readily be a sample from the distribution of the second generation.

The third generation was descended from the higher class individuals of the second. Only large litters were raised in the third generation, which also placed it in the class of a selected population. Their averages excelled those of the second generation in body-weight, promptness of mating, and percentage of fertile matings. The decrease of litter-size persisted in this generation, in spite of the selection of large litters only. Based upon the observations of King *loc. cit.* and the writer, the slightly superior vigor of the third generation in growth may be ascribed to the fact that the majority of the animals composing it were born in the winter months. The second generation was born during the summer months. Rats born in December seem to grow most rapidly; those born during the months following appear to be handicapped to progressively increased extents until after July.

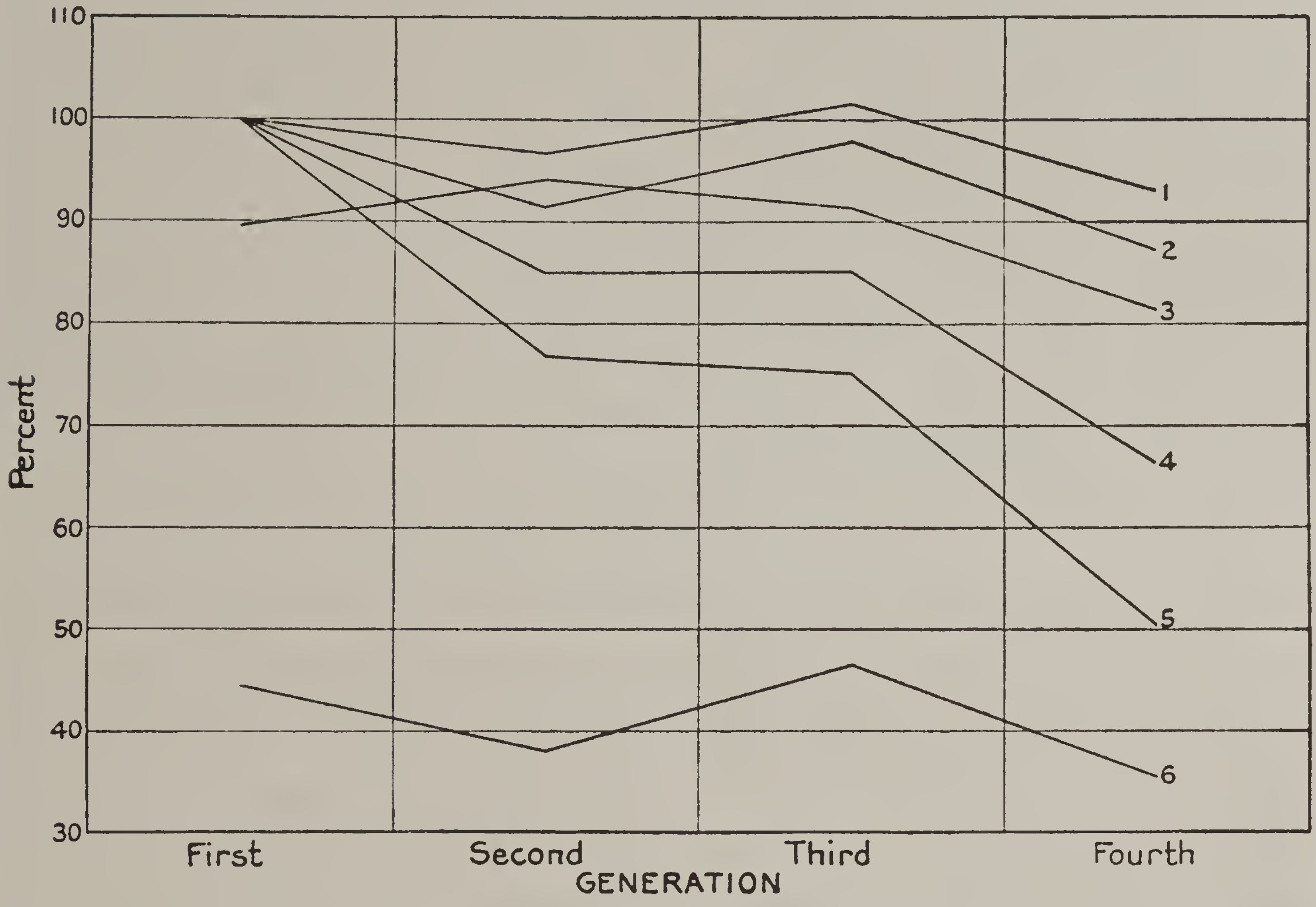

FIG. 4.—Behavior of characters of reproductive vigor and growth in successive generations of brother-sister matings.

1. Body-weight of females at 90 days of age in percentage of weight of the parental (not inbred) females.
2. Body-weight of males at 90 days of age in percentage of weight of parental (not inbred) males.
3. Percentage of live young born.
4. Percentage of fertile matings.
5. Average litter size in percentage of litter size of the parental (not inbred) generation.
6. Percentage of matings which were prompt.

The fourth generation was selected from the larger litters produced by the third. All characters of vigor were lower in this generation than in any of the preceding, with the exception of average length of time before matings became effective. This generation showed significant decreases in average size of litter, percentage of young born alive, and percentage of fertile matings. The causes of this marked decrease in the fourth generation are not clear. The animals were born during the fall months, which accounts perhaps for part of it. It is of interest to note that the average body-weights of the fifth inbred generation (which were offspring of the fourth) were higher than those of the fourth generation. This indicates that the low averages of the latter were not entirely genetic.

A survey of the results obtained after three generations of brother-sister matings indicates that the average vigor has decreased. This occurred notwithstanding a selection of members of large litters for parents of each succeeding generation. The decrease has tended to affect all characters which are dependent upon vigor. The average litter-size and percentage of fertile matings have been reduced most. An inspection of the results obtained in the cross-bred controls is interesting. In this stock the average size of litter also decreased from generation to generation. The frequency of stillbirths increased. The mating system employed with this stock, although it did not produce combinations of close relatives, seems to have been accompanied by a decrease in vigor of reproduction (fecundity). Growth and percentage of fertile matings were not similarly affected. The latter character tended to increase.

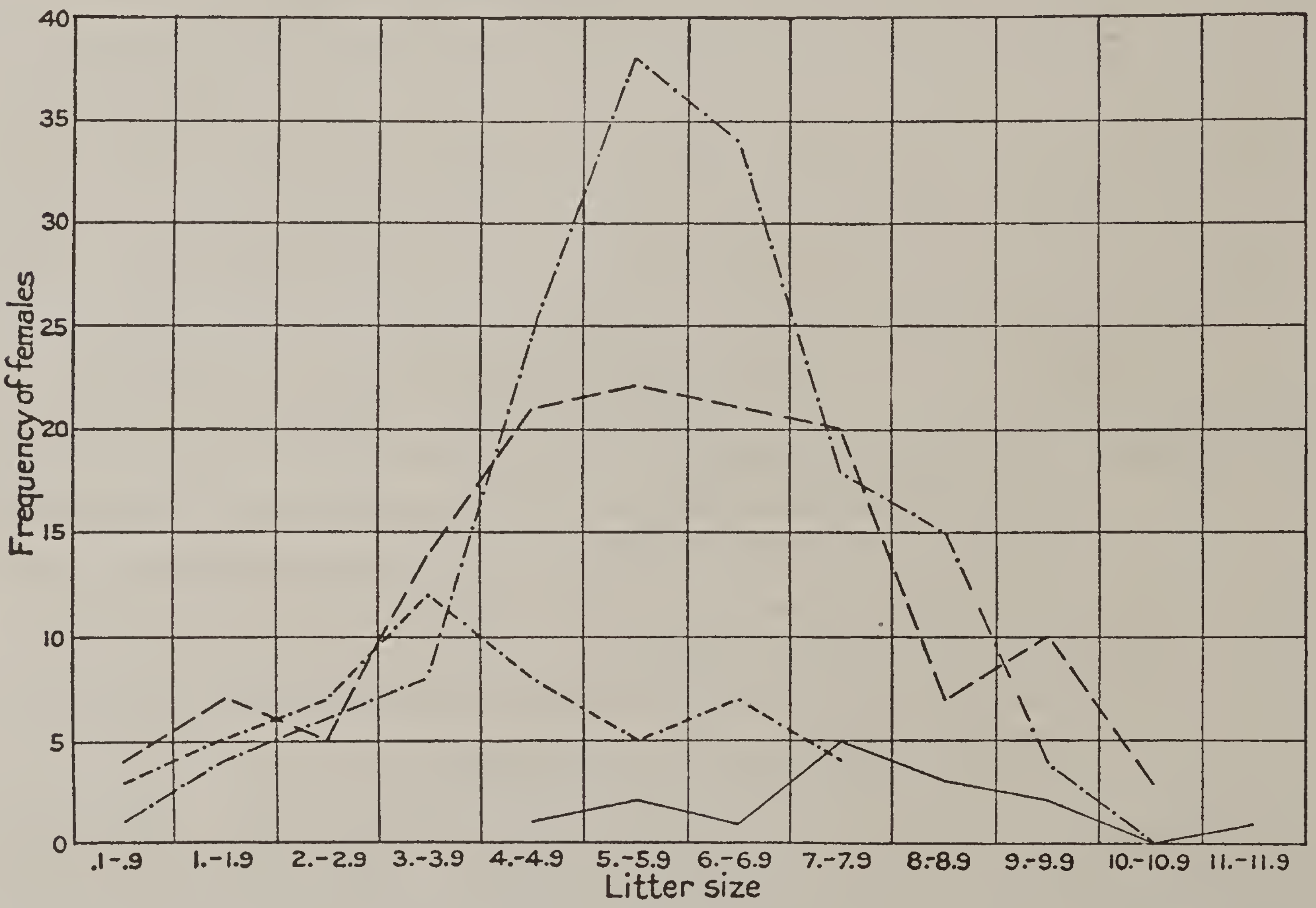

Fig. 5.—Distribution of females on basis of average size of litter. Solid line, mothers of first generation. Broken line, mothers of second generation. Dot and dash, mothers of third generation. Dot and two dashes, mothers of fourth generation.

The preceding remarks apply only to the averages of the inbred generations. In each of the three generations from brother-sister matings, individual rats and litters appeared which were at least equal to their ancestors of the first generation. An outstanding example was a male in the fourth generation of family B, which attained the weight of 535 grams at the age of 210 days. This was the heaviest rat observed thus far in this experiment. An unbalanced condition of the internal secretions may have contributed to this result, for he was 105 grams heavier than his heaviest brother at the same age. All of the characters observed presented wide variability in each generation.

The distribution of the females of the inbred stock is plotted in figure 5 on the basis of the average size of litter produced. The distribution is typical of each of the characters, with the exception of minor differences due to the environment. The variability as indicated by the "spread" of the curves is not significantly different in any of the four generations. The first and fourth are represented by smaller samples which give them a shorter range. It is to be noted that the modal class has shifted from 7 in the first generation to 5 in the second and third generations, and to 3 in the fourth generation. The "spread" of the third generation is noticeably less than that of the second. The distributions are symmetrical and form normal variation curves.

TRANSMISSION FROM GENERATION TO GENERATION OF REPRODUCTIVE VIGOR AND GROWTH.

Characters of vigor as manifested in growth and reproduction were extremely variable. From the tables and the foregoing discussion, it is obvious that they were influenced by various factors which were not hereditary. The phenotype was an extremely inadequate criterion of any particular genotype. There is no doubt, however, that part of the differences between individuals were genetic in nature.

The observations on other species have revealed evidence of clear-cut inheritance of differences in fecundity. Wentworth (1913) obtained three lines of *Drosophila* which produced distinctly different average progenies per pair. The F_2 generation from a cross between the extreme lines indicated that three pairs of genetic factors were responsible for the difference. Pearl (1912) concluded, from observations upon the winter egg production of Barred Plymouth Rock and Cornish Indian Game fowls and their crosses, that two pairs of genetic factors accounted for the differences. One of these appeared to be sex-linked. Simpson (1912) and Wentworth (1916) present evidence to show that fertility in swine fluctuates about three distinct means. Wentworth concluded that the differences were due to one pair of factors in which dominance was incomplete. Some hereditary factors which mendelize quite definitely are accompanied by decreased fertility. The dominant factor for yellow coat-color in the house mouse is always associated with low fertility and a tendency to become excessively fat but the low fertility in this case is known to be due to the lethal character of the gene for yellow. The vast majority of the differences in procreative power among the individuals of a species fail to yield readily to genetic analysis. These, like the differences in size, become "fixed," breed relatively true, and in crosses behave as blending characters.

The criteria of reproductive power used in the present study were size of litter, promptness of matings, percentage of young born alive and percentage of fertile matings. Growth was used as indicative of the degree of general vigor. The most reliable evidences obtained for the inheritance of these characters were: (1) The tendency of closely related fractions of each generation to exhibit reduced variability, compared to the population as a whole, for one or more of the characters; (2) the tendency of the offspring of individuals of such groups to possess similar average values of the characters and to show reduced variability. Average litter-size and weight at maturity (210 days) were examples. The three major groups of the population maintained characteristic individualities throughout the three later generations. Individuals descended from family A tended to be lowest in average size of litter and intermediate in body-size. Those from family B tended to excel in both average size of litter and body-weight at maturity. The descendants of family C tended to produce litters of intermediate size but were of smallest average weight at maturity. The tendency of the descendants of pairs of guinea-pigs to differentiate when inbred was observed to a marked degree by Wright, *loc. cit.*

The amount of resemblance between parents and offspring in characters of fertility and vigor was small. Tables 16 and 17 were arranged to show the degree to which females tended to resemble their dams in the average size of litter produced and in weight at 90 days of age. Table 18 shows the extent of the tendency of males to resemble their sires in weight at 90 days of age. These tables demonstrate that the following situations obtained: (1) Limited agreements between the grades of parent and offspring existed in these characters. High-grade animals tended to produce offspring which were above the average, and those of low grade tended to produce inferior progeny; (2) offspring tended to show average grades which deviated from the average less than the grades of their parent. A tendency toward filial regression existed. Closer agreements between the parents and offspring probably were prevented by the unknown hereditary contributions received from the other parent by the offspring in the case of each character.

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Table 1.—*Total number of young with age of mother.*

Generation of young.	Age of mother in days.									
	90–119	120–149	150–179	180–209	210–239	240–269	270–299	300–329	330–359	All ages.
First inbred	70	125	120	94	70	45	7	..	..	531
Second inbred	311	457	414	312	464	584	445	258	194	3,439
Third inbred	613	941	876	629	392	320	100	8	..	3,879
Fourth inbred	179	216	170	68	26	17	..	..	..	676
Fifty-second King inbred	51	40	40	15	..	..	..	..	..	146
First cross-bred	..	16	59	5	31	91	88	47	12	349
Second cross-bred	..	21	48	183	111	124	18	..	..	505
Third cross-bred	29	126	159	132	73	60	..	..	..	579
Total	1,253	1,942	1,886	1,438	1,167	1,241	658	313	206	10,104

Table 2.—*Total number of litters with age of mother.*

Generation of young.	Age of mother in days.									
	90–119	120–149	150–179	180–209	210–239	240–269	270–299	300–329	330–359	All ages.
First inbred	8	14	15	12	12	8	1	..	..	70
Second inbred	57	84	72	50	67	94	77	52	38	591
Third inbred	104	138	134	115	87	69	27	5	..	679
Fourth inbred	41	47	41	29	14	3	..	..	..	175
Fifty-second King inbred	9	7	5	3	..	..	..	..	..	24
First cross-bred	..	4	7	1	4	11	10	6	1	44
Second cross-bred	..	3	6	22	16	17	3	..	..	67
Third cross-bred	4	20	21	19	16	10	..	..	..	90
Total	223	317	301	251	216	212	118	63	39	1,740

TABLE 3.—*Average number of days from mating to parturition with age of mother.*

Generation of young.	Age of mother in days.									
	90–119	120–149	150–179	180–209	210–239	240–269	270–299	300–329	330–359	All ages.
First inbred	24	27	27	27	32	27	26	..	..	27.4
Second inbred	26	30	33	36	27	31	30	31	30	30.4
Third inbred	25	29	28	28	29	28	31	30	..	28.3
Fourth inbred	25	32	31	30	32	27	..	..	..	29.8
Fifty-second King inbred	27	31	28	26	30	..	..	..	..	28.2
First cross-bred	..	27	25	63	32	33	25	27	34	29.1
Second cross-bred	..	24	27	24	25	26	30	..	..	25.1
Third cross-bred	24	27	31	29	27	27	..	..	..	28.1
Average	25.2	29.5	30.0	29.8	28.3	29.4	30.2	30.6	30.4	29.0

TABLE 4.—*Percentage of delayed matings*[1] *with age of mother.*

Generation of young.	Age of mother in days.									
	90–119	120–149	150–179	180–209	210–239	240–269	270–299	300–329	330–359	All ages.
First inbred	12	57	53	75	67	63	..	..	..	55.7
Second inbred	37	65	82	66	45	60	70	69	70	62.9
Third inbred	11	51	56	63	71	61	81	60	..	53.1
Fourth inbred	27	72	76	79	87	66	..	..	..	64.6
Fifty-second King inbred	56	71	60	33	100	..	..	..	..	60.0
First cross-bred	..	50	14	100	100	18	40	50	100	40.9
Second cross-bred	..	..	50	5	12	35	33	..	..	19.4
Third cross-bred	..	35	81	74	62	40	..	..	..	57.8
Average	22.4	57.1	66.0	61.6	59.9	55.9	68.6	66.7	71.1	56.4

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[1] Delayed matings are those in which parturition did not result within 26 days after mating.

Table 5.—*Correlation of female's weight at 90 days with average number of days from mating to parturition.*

Average mating time.	Weight at 90 days in grams. 80–89	90–99	100–109	110–119	120–129	130–139	140–149	150–159	160–169	170–179	180–189	190–199	200–209	Total.
22–23..	..	..	..	..	..	2	1	..	..	..	1	..	..	4
24–25..	1	..	6	11	11	7	11	2	7	..	1	..	1	58
26–27..	..	2	6	16	18	19	18	7	5	1	1	2	..	95
28–29..	..	1	9	14	20	17	13	9	..	..	2	..	1	86
30–31..	..	2	5	9	13	13	7	5	1	1	..	2	..	58
32–33..	1	1	..	4	2	3	2	3	1	..	..	..	..	17
34–35..	1	4	..	4	9	8	..	1	..	..	1	..	..	28
36–37..	..	..	3	1	1	1	2	2	1	..	..	..	..	11
38–39..	..	..	2	2	1	..	..	..	..	..	..	..	..	5
40–41..	1	..	1	..	..	1	..	..	..	..	..	..	..	3
42–43..	..	2	..	3	..	1	..	1	..	..	..	..	..	7
Total:	4	12	32	64	75	72	54	30	15	2	6	4	2	372

Mating time: Mean, 29.48 days; S.D., 3.96 days. Weight: Mean, 131.30 gms.; S.D., 20.4 gms. Coefficient of correlation: —0.193 ± 0.033.

Table 6.—*Average size of litter with age of mother.*

Generation of young.	Age o mother in days. 90–119	120–149	150–179	180–209	210–239	240–269	270–299	300–329	330–359	All ages.
First inbred	8.7	8.9	8.0	7.8	5.8	5.6	7.0	..	..	7.59
Second inbred	5.5	5.4	5.7	6.2	6.9	6.2	5.8	5.0	5.1	5.82
Third inbred	6.0	7.0	6.5	5.5	4.5	4.6	3.7	1.6	..	5.71
Fourth inbred	4.4	4.6	4.1	2.3	1.9	5.7	..	..	..	3.86
Fifty-second King inbred	5.7	5.7	8.0	5.0	..	..	..	..	..	6.08
First cross-bred	..	4.0	8.4	5.0	7.7	8.3	8.8	7.8	12.0	7.93
Second cross-bred	..	7.0	8.0	8.3	6.9	7.3	6.0	..	..	7.54
Third cross-bred	7.2	6.3	7.6	6.9	4.6	6.0	..	..	..	6.43
Average	5.60	6.13	6.27	5.73	5.40	5.85	5.58	4.97	5.28	5.80

TABLE 7.—*Weight of female at parturition with size of litter.*

	Age in days.						
Litter size.	90–119	120–149	150–179	180–209	210–239	240–269	All ages.
1	158(5)	167(8)	187(5)	183(4)	175(1)	......	172(23)
2	157(3)	151(8)	165(5)	209(4)	217(2)	270(1)	176(23)
3	146(6)	162(5)	198(3)	198(5)	170(2)	225(1)	175(22)
4	157(6)	189(5)	210(1)	160(1)	243(3)	250(1)	190(17)
5	152(4)	174(7)	181(8)	180(3)	218(5)	......	182(27)
6	149(7)	192(6)	199(5)	209(4)	220(1)	240(1)	188(24)
7	167(6)	177(8)	181(6)	215(3)	200(1)	......	181(24)
8	174(4)	185(5)	192(8)	202(3)	......	217(4)	193(24)
9	158(5)	202(3)	214(4)	212(4)	250(2)	260(1)	203(19)
10	230(1)	205(5)	209(4)	242(2)	......	......	215(12)
11	......	198(5)	207(2)	230(1)	220(1)	......	206(9)
12	......	177(2)	225(2)	......	......	220(1)	211(5)
13	......	......	195(1)	220(1)	......	......	207(2)
14	......	......	210(1)	......	......	......	210(1)
Average	165(47)	179(67)	193(55)	198(35)	217(18)	254(10)	189(232)

Notes: Records of 81 females are included in this table.
Figures in parentheses indicate the frequencies of litters.

TABLE 8.—*Seasonal variations of reproductive vigor.*

Month.	No. of litters.	Total young.	Average litter size.	No. of females.	Litters per female.
Jan.	155	1014	6.5	167	0.93
Feb.	152	953	6.3	204	.75
Mar.	155	975	6.3	202	.77
Apr.	100	647	6.5	271	.37
May	164	1048	6.4	250	.66
June	207	1515	7.3	215	.96
July	172	1162	6.8	206	.83
Aug.	143	885	6.2	248	.58
Sept.	151	878	5.8	276	.55
Oct.	97	509	5.2	259	.35
Nov.	41	225	5.5	218	.19
Dec.	48	284	5.9	184	.26

Note: Data from April 1, 1923, to June 1, 1925 inclusive.

TABLE 9.—*Correlation of female's weight at 90 days with average size of litter.*

	Weight at 90 days in grams.													
Average litter.	80–89	90–99	100–109	110–119	120–129	130–139	140–149	150–159	160–169	170–179	180–189	190–199	200–209	Total.
.1–1.	..	..	2	3	1	2	..	1	..	..	..	..	..	9
1.1–2.	..	1	2	1	3	3	4	..	1	..	..	..	..	15
2.1–3.	1	3	3	4	3	3	1	..	..	..	..	1	..	19
3.1–4.	..	2	5	9	9	6	3	..	..	..	..	..	..	34
4.1–5.	3	..	7	10	16	7	9	5	2	..	..	1	..	60
5.1–6.	..	4	8	10	16	13	12	3	2	..	..	1	..	69
6.1–7.	..	..	4	14	12	17	10	7	4	1	..	1	..	70
7.1–8.	..	1	1	6	5	19	10	2	4	..	3	..	..	51
8.1–9.	..	..	1	5	3	2	3	5	3	..	1	..	..	23
9.1–10.	..	..	..	3	4	2	..	5	..	1	1	..	..	16
10.1–11.	..	..	..	..	..	..	1	1	..	..	1	..	1	4
11.1–12.	..	..	..	..	..	..	1	1	..	..	..	..	..	2
12.1–13.	..	..	..	..	..	..	1	..	..	..	..	..	..	1
Total:	4	11	33	65	72	74	55	30	16	2	6	4	1	373

Litter size: Mean, 5.73 rats; S.D., 2.14 rats. Weight at 90 days: Mean, 131.30 gms.; S.D., 20.4 gms. Coefficient of correlation: + 0.356±0.030.

TABLE 10.—*Frequency of stillbirths in percentage of total young with age of mother.*

	Age of mother in days.									
Generation of young.	90–119	120–149	150–179	180–209	210–239	240–269	270–299	300–329	330–359	All ages.
First inbred	2.9	3.2	8.3	5.3	24.3	37.7	..	..	..	10.38
Second inbred	6.4	2.8	4.3	2.6	3.7	5.1	9.0	14.7	10.3	6.08
Third inbred	10.1	7.5	6.9	10.9	6.8	12.2	11.0	25.0	..	8.77
Fourth inbred	16.7	11.6	16.4	33.8	57.7	29.4	..	..	..	18.64
Fifty-second King inbred	31.4	2.5	..	33.3	..	..	..	..	..	15.06
First cross-bred	..	..	3.4	..	9.7	..	..	2.1	..	1.72
Second cross-bred	..	..	4.2	2.2	13.5	.8	..	..	..	4.35
Third cross-bred	27.6	16.7	15.7	18.2	20.6	13.3	..	..	..	17.61
Average	11.01	6.95	7.69	9.57	9.25	8.06	7.69	13.09	9.70	8.66

Table 11.—*Frequency of litters containing stillbirths in percentage of total litters with age of mother.*

Generation of young.	Age of mother in days.									
	90–119	120–149	150–179	180–209	210–239	240–269	270–299	300–329	330–359	All ages.
First inbred	25.0	21.4	20.0	8.3	25.0	50.0	..	..	..	22.8
Second inbred	14.0	10.7	6.9	2.0	13.4	17.0	24.7	28.4	23.7	16.0
Third inbred	22.1	21.0	20.2	35.7	27.6	36.2	47.8	100	..	27.6
Fourth inbred	31.7	29.8	43.9	62.1	64.3	33.3	..	..	..	42.3
Fifty-second King inbred	77.7	28.6	..	33.3	100.0	..	..	..	..	45.8
First cross-bred	..	25.0	15.7	..	25.0	..	..	16.6	..	9.0
Second cross-bred	..	..	16.6	9.1	31.4	5.8	..	..	..	13.4
Third cross-bred	75.0	55.0	61.9	42.1	50.0	50.0	..	..	..	53.3
Average	25.1	21.7	22.5	29.1	27.7	24.5	27.1	41.3	23.0	25.57

Table 12.—*Growth of females.*

Generation.	Age of females in days.											
	30–	50–	70–	90–	120–	150–	180–	210–	240–	270–	300–	330–
Mothers of—												
First inbred	..	78(15)	113(15)	127(15)	178(15)	..	..	..	..	..	..	..
Second inbred	33(142)	74(135)	95(90)	123(128)	155(14)	161(7)	193(12)	184(34)	188(41)	209(24)	..	..
Third inbred	40(162)	76(162)	106(162)	129(162)	..	..	..	265(3)	212(15)	200(39)	197(29)	229(12)
Fourth inbred	37(63)	63(63)	96(63)	118(63)	166(47)	177(41)	190(25)	192(9)	207(2)	..	..	..
Fifth inbred	43(24)	81(17)	..	121(24)	..	..	..	..	..	..	..	..
First cross-bred	..	..	..	..	..	..	191(4)	196(8)	..	177(2)	..	..
Second cross-bred	53(6)	74(25)	102(25)	..	159(6)	167(19)	..	..	..	..	..	..
Third cross-bred	50(12)	85(20)	118(20)	142(20)	182(20)	202(20)	214(20)	221(16)	230(10)	..	..	..
Fifty-second King inbred	53(11)	105(11)	159(11)	190(11)	248(7)	247(3)	..	..	..	..	..	..
Fifty-third King inbred	46(41)	106(33)	..	157(40)	..	..	..	..	..	..	..	..
Average	38(461)	76(481)	106(386)	129(463)	174(109)	183(90)	201(61)	197(70)	200(68)	202(65)	197(29)	229(12)

Note: Numbers in parentheses indicate the number of rats weighed.

TABLE 13.—*Growth of males.*

Generation.	Age of males in days. 30	50	70	90	120	150	180	210	240	270	300	330
Fathers of—												
First inbred	..	92(14)	145(14)	171(14)	233(14)	..	..	..	..	..	..	..
Second inbred	36(133)	84(128)	122(86)	157(124)	204(9)	204(5)	242(12)	253(27)	266(57)	252(28)	..	..
Third inbred	42(113)	84(113)	131(113)	167(113)	..	..	..	270(3)	264(9)	259(27)	283(29)	266(11)
Fourth inbred	37(49)	69(49)	113(49)	149(49)	..	..	..	..	..	..	..	..
Fifth inbred	44(21)	106(12)	..	158(21)	..	..	..	..	..	..	..	..
First cross-bred	..	..	..	..	..	..	275(4)	271(7)	..	230(2)	..	..
Second cross-bred	52(5)	83(24)	125(24)	..	222(5)	234(19)	..	..	..	..	..	..
Third cross-bred	55(12)	96(20)	140(20)	176(20)	..	..	..	..	..	..	..	..
Fifty-second King inbred	52(11)	106(11)	174(11)	228(11)	..	..	..	..	..	..	..	..
Fifty-third King inbred	48(44)	122(37)	..	207(44)	..	..	..	..	..	..	..	..
Average	39(388)	82(408)	149(317)	168(396)	221(28)	228(24)	250(16)	257(37)	265(66)	254(57)	283(29)	266(11)

Note: Numbers in parentheses indicate the number of rats weighed.

TABLE 14.—*Variability of growth in females and males.*

Age in days.	Sex.	Weight in grams. 20–39	40–59	60–79	80–99	100–119	120–139	140–159	160–179	180–199	200–219	220–239	240–259	Number.	Mean.	Standard deviation.	Coefficient of variation.
30	Female	166	140	7	..	..	..	..	..	..	..	..	..	313	34.98	9.0	25.7
	Male	116	132	9	2	..	..	..	..	..	..	..	..	259	36.87	9.5	25.7
50	Female	5	39	132	127	26	..	..	..	..	..	..	..	329	73.37	16.2	22.0
	Male	3	24	61	121	63	14	..	..	..	..	..	..	286	82.87	19.5	23.5
70	Female	..	1	15	92	108	61	6	1	..	..	..	..	284	101.41	18.2	17.9
	Male	..	3	5	21	53	53	60	32	6	..	..	..	233	126.73	32.5	25.6
90	Female	..	..	1	14	82	131	85	20	4	..	..	..	337	126.29	19.7	15.6
	Male	..	..	1	3	8	35	73	62	57	32	7	2	280	162.57	25.9	15.9

TABLE 15.—*Summary of data concerning sterility.*

Generation.		Total matings.	Fertile matings.	Sterility due to male.	Sterility due to female.	Sterility undetermined.	Total males.	Total females.	Sterile males.	Sterile females.
Parents of—										
First inbred	Number	63	63	..	..	..	14	15	..	..
	P. ct. of total	..	100.0	0.	0.	0.	..	..	0.	0.
Second inbred	Number	483	411	18	51	3	132	151	4	6
	P. ct. of total	..	85.1	3.7	10.6	.6	..	..	3.0	4.0
Third inbred	Number	523	447	3	71	2	114	161	1	6
	P. ct. of total	..	85.4	.6	13.5	.4	..	..	.9	3.7
Fourth inbred	Number	190	126	11	48	5	49	62	1	11
	P. ct. of total	..	66.3	5.7	25.4	2.6	..	..	2.0	18.0
Fifty-second King inbred	Number	20	15	2	1	2	11	11	1	1
	P. ct. of total	..	75.	10.	5.	10.	..	..	9.1	9.1
First cross-bred	Number	47	41	..	3	3	13	14	..	..
	P. ct. of total	..	87.2	0.	6.4	6.4	..	..	0.	0.
Second cross-bred	Number	25	22	2	1	..	24	25	2	1
	P. ct. of total	..	88.	8.	4.	0.	..	..	8.	4.
Third cross-bred	Number	94	85	..	9	..	20	20	..	..
	P. ct. of total	..	90.5	0.	9.5	0.	..	..	0.	0.
Total	Number	1445	1210	36	184	15	377	459	9	25
	P. ct. of total	..	83.8	2.5	12.7	1.0	..	..	2.4	5.4

TABLE 16.—*Dam with daughter correlation of average litter size.*

		Distribution of daughters' average litter.												
Dams' average litter .1–1.	No. of dams.	.1–1.	1.1–2	2.1–3	3.1–4	4.1–5	5.1–6	6.1–7	7.1–8	8.1–9	9.1–10	10.1–11	11.1–12	Daughters' aver.
1.1–2	1	..	..	..	1	2	1	..	..	..	..	..	..	4.5
2.1–3	2	..	..	1	1	2	1	1	..	..	..	..	..	4.5
3.1–4	1	..	2	1	..	..	..	..	..	..	..	..	..	1.8
4.1–5	5	..	1	..	2	4	3	2	7	1	..	..	..	5.8
5.1–6	9	2	3	4	4	6	5	6	3	1	..	..	1	4.7
6.1–7	13	..	2	1	7	11	9	12	10	4	2	..	..	5.8
7.1–8	17	10	5	6	10	19	21	19	11	8	..	..	..	4.9
8.1–9	10	2	3	4	6	4	15	11	3	3	5	1	..	5.5
9.1–10	5	..	..	1	3	6	9	7	4	3	4	2	..	6.4
10.1–11	..	..	..	..	..	..	..	..	..	..	..	..	..	..
11.1–12	1	..	..	..	..	..	1	4	4	2	3	..	..	7.6
Total	64	14	16	18	34	54	65	62	42	22	14	3	1	

TABLE 17.—*Dam with daughter correlation of weight at 90 days of age.*

		Daughters' weight in grams.										
Dams' weights	No. of dams.	80–89	90–99	100–109	110–119	120—129	130–139	140–149	150–159	160–169	170–179	Daughters' av.
90–99	3	..	..	2	3	4	2	1	..	..	..	122
100–109	4	1	1	5	3	..	4	3	..	..	..	119
110–119	18	3	6	11	22	23	14	10	4	4	..	124
120–129	12	1	2	10	9	10	14	6	1	..	..	123
130–139	10	1	1	3	9	10	14	10	3	2	..	130
140–149	7	1	2	..	10	12	9	6	6	2	..	131
150–159	4	..	..	1	..	2	3	3	6	3	..	145
160–169	3	..	2	1	2	5	4	1	2	..	..	126
170–179	1	..	1	..	..	..	..	2	2	2	..	146
180–189	1	..	..	..	1	..	1	3	5	..	1	149
Total...	63	7	15	33	59	66	65	45	29	13	1	

TABLE 18.—*Sire with son correlation of weight at 90 days of age.*

		Sons' weights in grams.														
Sires' weights	No. of sires.	90–99	100–109	110–119	120–129	130–139	140–149	150–159	160–169	170–179	180–189	190–199	200–209	210–219	220–229	230–239	Sons' av.
90–99........	1	..	..	..	..	..	1	..	..	1	..	1	..	..	..	..	173
100–109.......	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
110–119.......	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
120–129.......	1	..	..	..	..	..	..	..	..	..	..	2	..	1	..	..	202
130–139.......	4	..	1	2	5	4	2	2	1	..	..	..	..	..	..	..	136
140–149.......	3	..	..	..	..	1	1	..	2	1	1	..	2	1	..	..	177
150–159.......	21	..	..	2	3	11	16	15	5	8	5	3	3	3	..	..	159
160–169.......	13	4	1	1	2	5	5	4	4	2	1	1	7	1	1	..	157
170–179.......	10	..	..	..	..	2	2	2	4	6	9	6	..	1	2	..	179
180–189.......	4	..	..	1	..	2	1	1	2	2	1	2	1	..	..	..	165
190–199.......	3	..	..	..	..	2	1	1	1	2	2	2	..	..	..	..	168
200–209.......	11	..	2	1	2	..	5	10	5	9	5	5	7	..	..	1	170
210–219.......	4	..	2	3	..	1	1	4	2	1	3	..	1	..	..	..	151
Total......	75	4	6	10	12	28	35	39	26	32	27	22	21	7	3	1	

TABLE 19.—*Comparative ranking of subgroups of the population in characters of vigor and fertility.*

Generation.	Average weight of males at 90 days.	Average weight of females at 90 days.	Average size of litter.	Percentage of still-births.	Average length of mating time.	Percentage of fertile matings.
Parents of—						
First inbred	3	4	2	5	2	1
Second inbred	5	5	6	3	8	6
Third inbred	4	3	7	4	5	5
Fourth inbred	6	6	8	8	7	8
Fifty-second King inbred	1	1	5	6	4	7
First cross-bred	..	..	1	1	6	4
Second cross-bred	..	..	3	2	1	3
Third cross-bred	2	2	4	7	3	2

PART III

THE JAPANESE WALTZING MOUSE: ITS ORIGIN, HEREDITY AND RELATION TO THE GENETIC CHARACTERS OF OTHER VARIETIES OF MICE

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With six plates.

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THE JAPANESE WALTZING MOUSE: ITS ORIGIN, HEREDITY AND RELATION TO THE GENETIC CHARACTERS OF OTHER VARIETIES OF MICE.

INTRODUCTION.

This experimental study of heredity was undertaken primarily for the purpose of testing the linkage relationships of the genetic factor for waltzing with various other known Mendelian factors in mice. As time went on, other interesting problems developed, such as the origin of the waltzer and the association of parental groups of characters.

With the rediscovery of Mendel's laws of heredity in 1900, many attempts were made to test the validity of these laws on all types of organisms. The result was the hybridization of many varieties of plants and animals, in the course of which some cases were observed which apparently did not conform to the simple laws of heredity. The first such case was reported by Bateson and Saunders (1906) in which sweet peas possessing purple color and long pollen grains were crossed with those having red color and round pollen grains. The F_1 generation was then crossed back to the double recessive, red-round. According to Mendelian expectation, there should result four types of individuals in *equal* numbers, namely: purple-long, purple-round, red-long, and red-round. Actually, however, a ratio was obtained approximating 7 : 1 : 1 : 7. Later aberrant ratios were observed in other crosses. Finally, Bateson and Punnett (1911) proposed a possible explanation by supposing that in gametogenesis of the germ-cells, those cells carrying certain characters were *reduplicated,* that is, divided more than the usual twice in the production of the mature gametes. However, these same observers encountered a serious objection to this theory, namely, that when the characters entered the cross from other combinations there was an apparent reduplication of other cells. For example, if the characters AB were crossed with ab (allelomorphs of AB) and the F_1 generation back-crossed to an ab individual, there resulted an aberrant ratio of xAB : 1Ab : 1aB : xab, where equality is to be expected; but where the characters Ab were crossed with aB and then the F_1 back-crossed to ab, the observed ratio was 1AB : xAb : xaB : 1ab, where equality is to be expected.

Similar phenomena were later observed by Morgan and his associates who realized that the reduplication hypothesis only partly explained the facts. Therefore, Morgan (1911) proposed the theory that such results could be accounted for by the supposition that certain factors were located in a single chromosome, and that each chromosome tended to act as a unit in heredity. All factors carried in the same chromosome would then show a tendency to associate together or form what are now called linkage groups. Characters which show strong linkage are supposed, according to this hypothesis, to be close together, while those that show weak association are widely separated on the chromosome. Moreover, on this hypothesis, characters which show *coupling* when introduced into a cross by the same parent, should show an equal degree of *repulsion* when introduced by different parents. This fact, observed by Bateson and Punnett but not explained by the reduplication hypothesis, is fully accounted for by the chromosome hypothesis. The latter has now been generally accepted as the correct and adequate explanation of linkage phenomena, even by the authors of the reduplication hypothesis.

It follows, as a necessary consequence of the chromosome theory, that the number of linkage groups should not be greater than the number of chromosomes. This relation has been clearly demonstrated by Morgan and his associates in certain species of *Drosophila.*

Since that demonstration, there has been a general interest in the subject of linkage, not only with reference to what characters are associated, but also as to how close is the association. The strength of association is measured by the percentage of cross-overs between any two characters. By cross-over is meant the dissociation of two characters, A and B, originally associated in the same gamete, and their reappearance in association with a new partner, as A with b or a with B. If A and B are independent characters, A is as likely to reappear with B as without it; that is, cross-overs will approximate 50 per cent of all cases. But if A and B are linked with each other, cross-overs will be less than 50 per cent, and the AB class will be larger than the Ab, the deviation depending on the strength of linkage.

With few exceptions, all of the better-known characters of mice, which have a simple Mendelian behavior in heredity, have already been tested against each other for their possible linkage relationships. One of the exceptions is that of waltzing, or dancing, as found in the so-called Japanese waltzing, or dancing, mouse. No systematic attempt has hitherto been made to find the possible genetic association of this character with respect to the mutant characters of the house mouse.

MUTATIONS OF THE HOUSE MOUSE.

The following characters of the house mouse, which follow the simple Mendelian rule in their heredity and which are therefore supposed to be due to the action each of a single gene or factor, have been reported and more or less fully tested for linkage: albinism, chinchilla, "extreme dilution" (Detlefsen's terminology, in this paper called *paleness* to distinguish it from the character long known as dilution), agouti, yellow, white-bellied gray, brown, dilution, black-eyed white, piebald, pink-eye, waltzing, various x-ray abnormalities, retinal abnormality, short-ears, sex-linked lethal, sable, black and tan, taillessness, bent or kinky-tail, and hairlessness, also single and multiple factor immunity characters.

Table 1 gives the more important reported data on the linkage relationships of these characters, as far as they have been tested. Many minor tests of association involving the same mutations have been made by various experimenters, such as Bateson (1903), Cuénot (1903–1911), Weldon (1907, 1917), Morgan (1908–1911), Durham (1908–1911), and others but, as these crosses involve small numbers, they are omitted.

Of all the characters mentioned above, no two have shown positive association with the exception of pink-eye in relation to albinism and its allelomorphs, although it is possible that further studies will show that linkage exists also between short-ears and kinky-tail.

AGOUTI.

Agouti is a characteristic color-pattern of the hair of many wild forms. It is a sort of "barring" or "ticking" of each individual hair, by means of which different colors are apparent in different regions of the hair. It, of course, can only manifest itself in the presence of some kind of color but may be inherited independently of any color, as in the albino which may carry agouti, but unexpressed in the absence of color. Agouti is always less pronounced in expression, sometimes absent, from the under side of the body. The entire absence of agouti (the non-agouti condition) gives the animal a uniform solid color, black or brown, a condition termed "self." The agouti pattern of the hair operates in such a manner as to change black to gray by breaking up the black of the hair with bands of yellowish or whitish color. The known allelomorphs of agouti, or characters completely linked with it, are white-bellied gray, yellow, and non-agouti. The first two are dominant over agouti. White-bellied gray, first reported by Cuénot (1907) and later by Morgan (1908), was found as a mutation in the wild form. Morgan considered it a type of white spotting and he was unable to reconcile its behavior with that of common recessive piebaldness. He says "it seems paradoxical" that spotting is at one time dominant and at another recessive. Later, Morgan (1911) reported a number of crosses of this form and established its position with respect to the other characters.

YELLOW.

Yellow was reported by Cuénot (1908) and others, and early crosses of this form produced puzzling results, from the fact that here was a color, not allelomorphic to other colors, and which because of its dominance might carry latent either black or brown or both. Cuénot (1908) first reported the non-conformity to Mendelian expectation of the inheritance of yellow and suggested its cause as the death of pure yellow zygotes. This hypothesis, the lethal character of homozygous yellow, has since been proven correct by Castle and Little (1910), Kirkham (1916, 1917, 1919), Ibsen and Steigleder (1917), Little (1917, 1919), and others, who find that the developing homozygous yellow embryos die and degenerate in the uterus.

While yellow has been reported as allelomorphic to agouti, its hereditary behavior could be equally well accounted for on the assumption that it is one of an independent pair of allelomorphs completely linked with agouti. A condition similar to this is described by Lippincott (1918, 1921) with respect to the blue Andalusian fowl, which he regards as a double heterozygote of extension and dilution, produced by mating a black, possessing extension and density with a blue splashed, possessing restriction and dilution. The dihybrid blues, mated *inter se*, give but three classes of offspring instead of the expected four, which, according to the above author, is due to complete linkage of extension with intensity, and restriction with dilution. This phenomena is explainable also by the much simpler hypothesis of Bateson, that of incomplete dominance of black. Bateson's idea is the one generally accepted.

The agouti series of multiple allelomorphs has been reported as independent of brown, dense, piebald, black-eyed white, pink-eye and albinism. Hagedoorn (1912), however, reports complete repulsion between agouti and color, but neither Dunn (1915) nor Little (1923), who have subsequently given particular attention to this question, are able to confirm Hagedoorn's conclusion, which may therefore be safely disregarded until additional evidence has been produced in its favor.

Dunn (1919), in one experiment reports an anomalous ratio between yellow and black, suggesting linkage. Little (1920), however, makes a quite valid remark that, since agouti and black have been shown to be independent, and yellow is allelomorphic to agouti, Dunn's ratio must have some other explanation, either random sampling or a dominant lethal linked with black.

BROWN.

Brown, commonly called chocolate, is a mutation due to the complete replacement of black-pigment granules with brown in the hair and eyes. It has been shown to be independent of dilution, piebald, black-eyed white, and possibly pink-eye (see Table 1). Miss Durham (1911) obtained figures that seem to show slight linkage in the last instance. Little and Phillips (1913) (Table 1) also obtained results which tend to show linkage between brown and pink-eye to the extent of a deviation of 5.75 times the probable error. Yet Detlefsen and Roberts (1911), who used over three times as many individuals and got no association, think that the aberrant results of Little and Phillips must have been due to a high differential mortality. However, if there is such a thing as homology of factors in different species of animals, then we might expect to find some linkage between brown and pink-eye in mice, since Castle (1924) has observed association of brown and albinism in rabbits, and pink-eye is known to be linked with albinism in mice.

DILUTION.

Dilution is that character that reduces the pigmentation of the coat, not by modifying the color of the melanin granules, but by a peculiar bunching or grouping of these granules. This condition was first analyzed by Miss Durham (1908, 1911) and later by Hagedoorn (1912) and Little (1913). Miss Durham shows that the color of the pigment granules is unchanged, but by a reduction in their number and a grouping together, they produce a dilute condition of the hair. Dilute black appears "blue" or "maltese gray," while dilute brown becomes a taupe. This mutation should not be confused with the albino allelomorphs, which tend to make the color pale. Dilution, as shown in Table 1, has been tested by various authors against pink-eye and found to be independent. Other tests have been made against other known factors but, while they are not very significant on account of the small number involved, they tend to show independence.

SPOTTING.

There have been two types of spotting reported, one a dominant and the other a recessive. The former is the type found in the variety known as "broken" and associated with piebald in black-eyed whites. It was first reported by Durham (1908). The latter is the common piebald, first reported by Cuénot (1902). Little (1915), So and Imai (1919), and Dunn (1920) all report that these two types of spotting are independent of each other and due to different factors. The black-eyed white condition, however, they report as having usually associated with it the piebald factor, for when black-eyed whites are bred *inter se*, they regularly produce some piebalds.

That there are at least two different types of spotting seems certain. They can generally be distinguished by the manner in which they are expressed; the most common form of the dominant type appears as spots or patches of color, varying in size, yet characteristically small, on a white background; the recessive type may be described as generally a colored coat from which the pigment is absent in certain regions. In the former, the pigment areas are usually small, quite irregular, and scattered over any or all parts of the upper surface of the body. In the latter, the pigment areas are in large patches, occurring in more or less definite regions, particularly the shoulders and rump, and are seldom broken up into scattered spots. This type will grade from an almost wholly pigmented animal to one with only one or two spots on the ears, shoulders or rump. It is possible that certain phases of expression of each type may scarcely be distinguished from the other, yet, on the whole, the writer is inclined to believe that, in the absence of any selection, each form is readily distinguishable. The two types are represented in Plate 1, figure 1.

All experimenters who have worked with these characters are agreed on the following points: That the so-called black-eyed white mouse possesses both of these spotting factors; that in the homozygous condition the factor black-eyed white is lethal; and that the hypothetical, homozygous black-eyed white is a pure white animal with black eyes. This condition is seldom if ever realized, although Dr. L. C. Strong informs the writer that he has gotten such forms. De Aberle (1925) in a recent paper gives evidence for the death of the homozygous black-eyed white. He finds that the lethal individuals occur as anemic young which die shortly before or after birth.

The distribution of spotting over the body, at least in the recessive type, seems to be determined by independent modifying factors (Little 1917, Dunn 1920), two of which appear to have been isolated by Dunn and Durham (1925) in races characterized respectively by a white face and a belt.

As shown in Table 1, all authors report the two types of spotting as independent from all characters against which they have been tested.

The type involved in the writer's experiments is that found in the pure Japanese, which is the recessive form (Plate 1, fig. 1; Plate 5, figs. 1, 2). Its recessiveness, however, is not always complete, at least when crossed with some strains known to be homozygous for self.

PINK-EYE.

Pink-eye is an early described mutation from the normal pigmented eye (Darbishire, 1904; Cuénot, 1907; Durham, 1911) and represents a condition in which the eye is almost, but not entirely, free of pigmentation, an eye condition similar to that found in the albino. It is a supplementary factor with respect to the coat-color; in other words, it modifies the coat-color, reducing black to a pale bluish-gray, denoted as lilac by Miss Durham. Its effect on brown, however, is not quite so great, yet it does cause a distinct color change—"chocolate lilac" of Miss Durham, "cafe au lait," of Cuénot.

This condition has been shown repeatedly to be associated in its heredity with the gene for albinism. As will be seen in Table 1, Darbishire (1904) obtained a deviation from the normal unassociated ratio of 26.5 ± 6.8; Castle (1919) of 563.5 ± 18.9; and Dunn (1920) of 563.2 ± 16.4 and again 581.2 ± 16.8. Haldane-Sprunt-Haldane report reduplication. Detlefsen (1923) reports linkage between pale color and pink-eye, but since this type of paleness is allelomorphic to albinism, we would most naturally expect association here also, and to the same degree.

ALBINISM.

Albinism is perhaps the oldest mutation to be mentioned in mice, as it likewise is about the first to be reported among other animals. In mice it is shown in its completeness where no pigmentation is visible on any part of the body or in the eyes. Besides full color, two other allelomorphs to albinism have been reported. The darker one was called Chinchilla by Schuster (1905), "blasser" by Plate (1910). It is also reported by Hurst (1911), Hagedoorn (1912), and Feldmann (1922). It is a factor that tends to lighten the coat-color but slightly, having little effect on black or brown, but reducing yellow to practically a white, as in the chinchilla rabbit. Hagedoorn reports this gene as producing a "fade color."

The other allelomorph in the series, "a dilute allelomorph of albinism" (Detlefsen, 1921), reduces all coat-colors to a pale cream. To avoid confusion with the factor commonly called "dilution," it would seem preferable to designate this albino allelomorph as "pale." Any association involving albinism must, of course, include also its allelomorphs, full color, chinchilla and paleness.

SHORT-EAR.

Short-ears (fig. 2) is a mutation discovered by Miss Lynch (1921) in the stock of Miss Lathrop. The ears are considerably shorter and thicker than normal and, from specimens observed, the character seems to be associated with morphological variations of the head and skull. It behaves as a simple recessive to normal ear-length. So far as reported, it has not been tested against any other character for linkage, although the writer understands that Miss Lynch has some tests under way.

TAILLESS.

Another morphological mutation was early reported by Nägeli (1911) (see Lang 1914), in which the tail was either wanting or very much abbreviated. This mutation showed a dominant tendency when bred to normal, producing approximately equal numbers of normal and short tails, which apparently indicated a heterozygous dominant mutation. However, when two short-tailed individuals were bred together they produced 39.1 per cent of normal-tailed individuals. Apparently he was unable to obtain a homozygous short-tail, which may indicate a linked lethal factor or a condition similar to that of the yellow mouse.

KINKY-TAIL.

Crooked, or kinky, tail is another mutation described by Plate (1910) and later by Blanks (1916). This mutation expresses itself in the form of a crook or series of kinks which may be at any part of the tail, proximal, middle or distal (Plate 1, fig. 2). Apparently, from the reports of Plate, it is linked with pink-eye. He obtained 41 pink-eyed individuals with the mutation and only one dark-eyed. The behavior of this mutation is, like taillessness, eccentric. The data from his table show that he obtained 9 abnormal (bent tail) and 1 normal young, when both parents were abnormal; 19 normal and 10 abnormal young, when one parent was abnormal and the other an unrelated normal; 18 normal and 9 abnormal young, when both parents were normal but sibs to abnormal; 6 normal and 1 abnormal young, when both parents were normal but one was sib to abnormal; and 16 normal and 5 abnormal young, when one parent was abnormal and the other normal but sib to abnormal. These and other facts reported by Plate indicate that this character varies much in its expression, behaving sometimes as a dominant, sometimes as a recessive in relation to normal, but probably depending on a single gene.

RHINOCEROS MOUSE.

The "rhinoceros" mouse is another peculiar mutation in the house mouse, which consists in a hairless condition of the skin which is much wrinkled and folded in heavy pleats. The vibrissæ seem to be the only hairs present. It was first described by Gaskoin (1856), who states that the color was "pinkish white" with black eyes. He reported that the epidermal scales were normal and healthy, "examination of the surface and sections of the dermoid covering with high and low powers of microscope showed no trace of hair follicles or glandular bulbs." He was unable to breed these mice in captivity, but a pregnant female, "big with kit," was caught in the field and produced several young which he reported as being "perfectly naked from birth." In this respect these mice differed from a similar condition reported by Roberts (1924) in rats, in which the young do not lose the hair until about 3 weeks of age. Sumner (1924) reports a like condition in the deer mouse, *Peromyscus*. Roberts and Sumner both report this mutation as a simple Mendelian recessive, with low viability and high sterility.

X-RAY ABNORMALITIES.

Little and Bagg (1924) describe the inheritance of several structural abnormalities in the descendants of mice that had been treated with x-rays. Mutations in the form of abnormalities have occurred which affect various parts of the body, but particularly the head and eyes. They describe morphological malformations of the head, jaws, feet, and abnormal development of hair on the body forming a sort of "saddle" around the middle of the body. In other mice similarly treated with x-rays, Bagg (1924) finds that abnormalities of the kidney are associated with those of the eye, usually being found on the same side, if only one side is affected.

RODLESSNESS.

Keeler (1924) reports a retinal malformation, termed rodlessness, in a race of albino mice in which the three external retinal layers are suppressed. This mutation appears to be inherited in simple Mendelian fashion.

SEX-LINKED LETHAL.

There has been but one sex-linked character described in mice, and that is a supposed lethal described by Little (1920). He reports this as occurring in the Harvard strain of Japanese waltzers. He bases his conclusions on the smaller size of the litters and the excess of females in the pure-bred stock, neither of which peculiarities is found in outcross litters. The writer, however, who has bred this same strain of mice since 1920, finds that the sex-ratio in his experiments is normal, and he is inclined to believe that the smaller size of the litters is a racial character inherited from a separate wild species. It would seem probable, therefore, that the lethal factor of Little, if that is the correct explanation of the peculiar sex-ratios observed by him, was something which had originated within his own stock, since it is not found in another sample of the same inbred strain.

IMMUNITY.

In addition to the genetic factors affecting color and form, there are certain physiological immunity factors which undoubtedly play an important rôle in the natural life of mice. These factors appear to be quite exact in their reactions, immunizing one animal and rendering another susceptible, when morphologically they are so nearly identical that they can scarcely be distinguished. Tyzzer (1909), Cuénot and Mercier (1910), Little and

Tyzzer (1916) and Little and Strong (1925) have all reported on the inheritance of a susceptibility to carcinoma in mice. The susceptibility factors, in most cases, seem to be multiple, varying in number, however, from 1 to 5 according to the physiological type of carcinoma used and are usually dominant in character. Hagedoorn-LaBrand-Hagedoorn (1920) report a predisposition to a bacterial disease, staphylococcus, which behaves as a Mendelian dominant. This susceptibility was found in a race of imported Japanese mice similar to the waltzer, and may prove to be a possible explanation for the delicacy of both of these races.

Modifications of the yellow in mice have been reported by Miss Durham (1911), Plate (1918) and Dunn (1920), in the form of the so-called sables, along with which Dunn (1916–1920) would also include the black and tan. The latter apparently possess a darkening factor or factors which tend to intensify all coat-colors. Their exact analysis is difficult, for as Dunn remarks, "The results throughout indicate that we are dealing here with genetic factors similar to those which have produced such quantitative differences as size in the races of rabbits."

WALTZING MUTATION.

Waltzing is that condition quite completely described by Yerkes (1907) in his book on the "Dancing Mouse." This character was also known to many earlier writers—von Guaita (1898, 1900), Darbishire (1904), Cuénot (1908, 1911), Haacke (1895, 1906), Weldon (1907), Bateson (1909), Fortuyn (1912), Hagedoorn (1912), Hammerschlag (1912), Lang (1914), and others. Mice homozygous for the waltzing factor are unable to orient themselves in a horizontal plane, resulting in very erratic and rapid turning and whirling movements in circles sometimes wide, sometimes narrow, sometimes figure eights, all repeated many times in rapid succession. Movements of the head are quite characteristic and may be described as turning, twitching, jerking and swaying, "vertiginous" of Bateson. Waltzing mice are also totally deaf, the two conditions being correlated and due probably to the same morphological or physiological cause.

ORIGIN OF THE JAPANESE WALTZING MOUSE.

The mouse found more or less abundantly throughout Central Asia, which is probably the progenitor of the domesticated Japanese waltzing mouse, was described by Eversmann (1848) as follows: "Mus wagneri—M. supra caudaqua griseo-fuscus; subtus abrupte candidus; auriculis majusculis; verruca halucari lamnato; cauda quam corpus breviore." The author further comments on this description, explaining each item more fully.

> In color, this mouse approaches nearest to the darker varieties of *M. sylvaticus*, from which, however, it can be clearly distinguished by other characteristics. It is about four times smaller, smaller than even *M. minutus*, and hence one of the smallest mammals. The tail is considerably shorter than the body; the ears are comparatively longer and more hairy than those of *M. sylvaticus;* the thumb-warts of the fore-feet have a

distinct nail. The dorsal side of the body is of a grayish-brown color, with a tendency toward reddish-yellow; the ventral side as well as the feet are pure white; on the sides the two colors are rather sharply separated; the hairs of the under side are not bluish at their base as in *M. sylvaticus*, but white throughout. The tail has about 19 or 20 vertebræ and about 130 scale rings; it is nearly uniform in color, grayish-brown, both above and below, but somewhat lighter on the under side; it is somewhat more hairy than that of *M. sylvaticus*. The claws of all toes are white. (Translation from the original German.)

OPINIONS.

Bonhote (1905) makes the statement that Wagner's mouse is quite common throughout Central China, Mongolia and other central Asiatic regions, and that it occupies in man's life there a position similar to that occupied by the house mouse elsewhere. Even today *Mus musculus* is not at all abundant in those regions, if we may judge from the reports of travelers and the number of specimens collected by scientific expeditions.

What is said in the following paragraphs in regard to the origin of the dancing mouse will probably apply also to the Japanese race of mice reported by Hagedoorn, LaBrand, Hagedoorn (1920). These authors describe a mouse imported from Japan, a "very minute domestic mouse probably belonging to the same group as the commonly imported, oriental, waltzing mouse."

Several writers (Haacke (1895), Fortuyn (1912), Morgan (1911), Hagedoorn (1912)) have suspected that the waltzing mice may have had a different origin from the common fancy mice, and, as will be shown, there are both morphological and physiological facts to substantiate this belief. Morgan (1911) commenting on this says: "The supposed origin in China of the waltzing mice, the peculiar shape of the head, their size and proportions of the body, strongly suggest that they belong to a different race (species?) from that from which our common domesticated mice have arisen."

Fortuyn (1912) advances several reasons for thinking that the waltzing mouse has had a separate origin from the common fancy mouse: First, its point of origin, Central Asia, from whence it was carried through Japan to Europe, as suggested also by Yerkes (1907). Second, the fact that there is found in Central Asia a species of *Mus*, *M. wagneri*, which is quite abundant, and which resembles the waltzer in size and measurements, particularly in tail-length. He reports the average tail-length of the waltzer (11 individuals) as 52 mm., the average of the wild *M. musculus* (18 individuals) as 75 mm.; likewise the average number of scale rings on the tail in the waltzer as 136.9 (maximum 145, minimum 128), and in *M. musculus* as 197.0 (maximum 214, minimum 176). These figures differ slightly from measurements of corresponding stock in the Bussey Laboratory, as will be seen in Table 2. Third, Fortuyn states that the ratio of waltzing to normal individuals in the F_2 generation is abnormal. By combining the results of several experimenters he finds a ratio of 157 waltzers to 961 normals, or only 16 per cent waltzers where expectation is 25 per cent. This, however, is inconclusive as the writer obtains normal ratios in all outcrosses. Fortuyn is so confident that his observations indicate a derivation from this Central Asiatic species that he describes the waltzer as a new variety of *wagneri*, naming it *Mus wagneri*, var. *rotans*.

EVIDENCE FROM JAPANESE NETSUKÉ.

Schlumberger (1894) describes an ancient Japanese wood carving, of a group of waltzing mice. It consists of 2 adults, parents, and 8 young, compactly arranged. The various postures of the individuals of the group are similar to attitudes assumed by any group of mice in a nest. The author states that the detail of the carving is characteristically Japanese in its minuteness. The external sex-characters, scale rings of the tail, and other details are distinctly shown. Both parents and 4 young are represented as black and white spotted (Dutch type); of the other 4 young, 2 are white and 2 black. The present writer does not attempt to analyze the genetics of the group.

Mr. Dodge, curator of Japanese art, of the Boston Art Museum, has kindly identified the carving by means of the description and illustrations given by Schlumberger. Mr. Dodge states that it is a "netsuké" or toggle. Toggles are attached by a cord or chain to personal articles and serve to counterbalance such when suspended over the belt. They are chiefly ornamental. This particular toggle, identified by the artist's name on the under side, is the work of Masateru, an artist who specialized on small animals. His principal works deal with mice and other small domestic pets. He lived during the last part of the eighteenth and the first part of the nineteenth centuries.

These facts give a valuable clue to the history of Japanese mice. It is certain that they were well known as household pets at the beginning of the last century. Therefore, since the house mouse is not a native of China and was probably not introduced until the opening of that counrty to commerce about the middle of the nineteenth century, we can hardly escape the conclusion that the waltzing mouse is a domesticated variety of some native, central Asiatic form. Also that it has been known in its present form and color-pattern in China and Japan for a hundred years or more, at least many years before the house mouse was known to these people.

EVIDENCE FROM MEASUREMENTS.

The first five columns of Table 2 show a compilation of a series of measurements of *Mus wagneri*, the pure Japanese waltzer, the wild *M. musculus* (herein called "wild"), and the common fancy mouse. The fancy mouse used as a type in this table is of the well-known strain of albinos bred by Dr. H. J. Bagg of New York, and probably represents the average of the common varieties, at least structurally. Measurements 2 to 12 inclusive were suggested by the work of Hilzheimer (1911), and in part are taken from his paper and in part from the average measurements of 13 specimens collected by the Wulson Expedition, now in the Zoölogical Museum of

Harvard University; measurements 13 to 15 inclusive were similarly suggested by Thomas (1905); while the balance constitute a miscellaneous set taken to show certain peculiarities of each race. Among the first 16 are found the measurements used by systematists generally in distinguishing species and varieties. The others, not generally considered significant in classification, show obvious racial peculiarities. All measurements were taken with a vernier scale and are recorded in millimeters.

A comparative study of the domesticated varieties with the two wild forms can hardly leave any doubt as to the progenitor of each. An examination of measurements of *wagneri* and the waltzer shows a marked similarity between them and, with one exception, the differences are no more than one would expect in a domesticated variety of any wild form. Such is the case also when the wild and fancy are compared. Certain cranial peculiarities, which will be discussed later, are common to each group. On the other hand, comparison of *wagneri* with the wild, or Japanese with fancy, shows significant differences in practically every detail.

That the fancy should resemble the house mouse more closely is to be expected, for all breeders report frequent crosses with the wild. There has thus been repeated introduction of wild blood into the fancy varieties. In the case of the Japanese, however, entire absence of ancestral form, from those parts of the world where this race has been most intensely bred, would eliminate any possibility of preserving the wild characters by crossing back to the original type. *Mus wagneri* is limited in its range to Central Asia and is not found in any of the mountain or coastal regions, where most certainly the Japanese had been bred for a long time, even before its introduction into Europe. It is quite remarkable that the Japanese has retained so consistently its specific characters, in spite of long domestication.

The one exception to similarities between *wagneri* and the Japanese is the long skull of the latter. It is interesting to note, however, that this lengthening occurs only anterior to the posterior nares, with most of it in the region of the inter-dental gap. This space is 0.8 mm. longer in the Japanese than in *wagneri*. Measurements of the posterior portion of the skull are remarkably similar to *wagneri*. There is no cranial elongation posterior to the palatines as shown by the basilo-palatilar distance, 7.3 mm. and 7.4 mm. in *wagneri* and waltzer, respectively. Along with the lengthening of the skull, there is a slight, but hardly significant, narrowing. All measurements of width are slightly less than in *wagneri*.

Whether or not these changes are the result of domestication, or are a feature of the waltzing character, or both, can only be conjectured. The writer is inclined toward the second view, which is partly supported by negative evidence as seen in the fancy mouse. Here domestication has apparently had a tendency to widen and shorten the skull rather than lengthen it. The ratio of width to length is 52.0 per cent and 54.3 per cent in the wild and fancy, respectively; while in the *wagneri* and Japanese it is 58.3 per cent and 51.2 per cent, respectively. Note that there is a relative widening of over 2 per cent in the fancy, but a narrowing of 7 per cent in the waltzer (Plate 2, figs. 1 and 3).

A few other interesting conditions are noticeable in the skulls of these different races. One of these is the position of the posterior nares. In both the wild and fancy mouse the anterior edge of the opening is posterior to the line joining the posterior edges of the third cheek teeth alveoli; while in both the *wagneri* and Japanese it lies anterior to or, at the most, in line with it (Plate 2, figs. 2 and 4). The extent of this variation may be calculated by taking the sum of the seventeenth and eighteenth measurements and comparing it with the fifteenth. Note that in *wagneri* 17 plus 18 is greater than 15, in the waltzer it is equal; while in both wild and fancy 17 plus 18 is less than 15. Along with this, we find that the incisor alveoli are extended further posteriorly in both *wagneri* and Japanese than in the wild and fancy. Thus the palatine bridge becomes noticeably narrower in the former two than in the latter two. Dr. G. M. Allen of Harvard University informs the writer that such cranial peculiarities are of considerable significance in taxonomic relationships.

Another interesting condition is seen in the size of the foramen magnum (Plate 3, figs. 1 and 2). In the *wagneri* its two dimensions are 3.3 mm. and 3.7 mm., to which the Japanese closely approximates with 3.3 mm. and 3.5 mm.; in the wild, these dimensions are 3.6 mm. and 4.1 mm., as against 3.9 mm. and 4.4 mm. in the fancy. This gives an aperture approximately 26 per cent greater in the wild and 47 per cent greater in the fancy than that in the *wagneri* and Japanese.

The ratio of tail to total length is usually quite constant in any one species, and is frequently employed in classification. This in Wagner's mouse and the waltzer is 41.8 per cent and 41.3 per cent, respectively, showing a more striking similarity between these two forms than is manifest in the simple measurements of length. These ratios are significantly different from those of the wild and fancy, which are 46.2 per cent and 45.8 per cent, respectively.

A study of measurements 25 and 26 shows that the greater length of the tail is brought about by a lengthening of the tail vertebræ, and not necessarily by an increase in their number. Arbitrarily the tenth vertebra was taken as a type. This is 88 per cent and 100 per cent longer in the wild and fancy, respectively, than in *wagneri*, while in the Japanese it is but 37 per cent longer. This change produces a relatively longer tail in both of the domesticated varieties. Whether or not this is due to the influence of domestication can only be conjectured. But since somewhat similar conditions are apparent in rabbits where there is a general lengthening of body-measurements under domestication, the writer inclines to believe that a like situation exists in these mice. The waltzer has gained two tail vertebræ, possessing either 21 or 22 as compared with the *wagneri* 19 to 20. These numbers, however, are significantly different from the 26 and 28 found in both wild and fancy.

The number of scale rings of the tail likewise shows marked differences between the two species. In Wagner's mouse, Fortuyn gives an average of 136.9 which is quite close to the mean of the Harvard University specimens. In the Japanese, the number is 141.0 average, a very close approximation to *wagneri*, considering the two added vertebræ. In the wild and fancy the number of rings is 175.1 and 180.8 respectively, showing a striking difference between these and the former. While of course the width of rings varies in different parts of the tail, yet the average number per millimeter is significantly different in the two species: 2.3 and 2.4 in the Japanese and *wagneri*, respectively, and 2.0 in both wild and fancy.

On the whole, structural measurements of the four types of mice (*wagneri*, Japanese, wild, and fancy) show a striking and consistent similarity between the first two and the last two. This is even more impressive when one considers the history of domestic mice and the probabilities of hybridization. While it is true that European mouse fanciers are very careful in breeding their pure races, we can hardly imagine a race as old as this, and which breeds as freely with the fancy mouse, into which some foreign blood has not been introduced at some time or other.

EVIDENCE FROM ANATOMICAL STRUCTURE.

Eversmann in his original description seems to stress the existence of the thumb nail in *M. wagneri*, the distinctness of which appears to him to be quite significant. Hilzheimer (1911), however, commenting on Eversmann, is quite skeptical about the "distinct nail," and even goes so far as to say that he is unable to make it out. The present writer has examined a number of specimens in the Zoölogical Museum at Harvard University and finds this nail very inconspicuous, in fact, it can only be seen with the aid of a hand lens.

The word "nagel" used by Eversmann has been translated as "nail" rather than claw, for it is a broad, flat structure, quite unlike the claws of the other toes, but similar in appearance to the primate nail. It is doubtful whether or not this was the author's meaning.

In comparing the thumb nail of the Japanese waltzer with that of the wild house or the fancy mouse, we find that in spite of the much smaller body-size, the nail of the waltzer is actually larger. In the waltzer, the nail is 1.02 mm. long and 0.97 mm. wide; while in the fancy mouse its dimensions are 0.76 mm. by 0.72 mm. Whether or not these differences are significant could be shown only by a further comparative study of this structure in other species of *Mus*. They do, however, lend support to other facts indicating origin of the Japanese mouse from *M. wagneri*.

Several authors, Bateson (1903) and others, have suggested that there may have been an outcross of the fancy mouse with the wild, western European species, *Mus sylvaticus*, and possibly also with *M. minutus*, but from body-measurements reported by Barrett-Hamilton (1900) there is no indication of such crosses having been made. Most certainly these two wild forms, and particularly *M. minutus* on account of its small size, have not contributed toward the present race of so-called Japanese mice.

The evidence from anatomical structure, therefore, would of itself fully justify the placing of the Japanese waltzer in the species *Mus wagneri*.

EVIDENCE FROM EYE PIGMENTATION.

As will be described, the Japanese possesses peculiar eye pigmentation, in that the melanin granules are almost entirely absent from the choroidal layer of the eyeball. It was thought that possibly this might be a clue to further evidence of the derivation of the waltzer from *wagneri*, if the latter proved to possess this character. Therefore, through the kindness of Dr. G. M. Allen of Harvard University, the writer obtained four eyes from dried specimens of *M. wagneri*, collected by the Wulson expedition in Mongolia. Sectioning of these eyes was exceedingly difficult on account of their dehydrated condition. In drying, the cell-tissues of the eyeball had become practically cemented to the lens and it was found impossible to separate them. Consequently, it was necessary to section the eye as a whole. The lens was so hard that in attempting to section it, it would merely nick the edge of the microtome knife and break, as if it had been a glass crystal. Two eyes were ruined before a method of sectioning was developed. Finally it was found that sections could be cut by mounting the eye between cork and using a solution of shaving soap to moisten and soften the material; then by placing the microtome knife at an angle and keeping it wet with the same solution, sections approximately 10 μ could be cut with some degree of success.

An examination of these sections was disappointing, as it was found impossible to differentiate between the layers of tissue. In places, what appeared to be the pigment layer was quite thin, indicating the possibility of only one pigment layer. But when the eyes of the common mouse were similarly treated by drying and sectioning, it was found that all the tissues of the eyeball, in shrinking around the lens, become stretched in parts and wrinkled in others, producing in places very thin layers and in others quite thick. This fact would thus discredit any conclusion that might be drawn from the thickness of the pigment in the eye of Wagner's mouse.

However, the iris showed a condition which might well be interpreted as indicating a retinal pigment layer only, as a similar condition exists in those varieties that have only this layer. Normally the two pigment layers of the eyeball are continued around and into the iris; the choroidal layer forming the front and the retinal the back of the iridal layers. Throughout most of the iris these merge one into the other and there is no line of separation, but near the pupil they are distinctly divided by the sphincter muscle which lies between them. In plate 3, figures 3 and 4 show, respectively, the condition when both and when only one layer is pigmented. In the first there is pigment in front of and behind the sphincter; in the second, only back of this muscle.

Figures 1 and 2 of Plate 4 are photomicrographs of a section of the eye of *wagneri* showing a part of the iris immediately surrounding the pupil. It is clearly evident that there is an absence of pigment in front of the sphincter muscle; yet normal pigmentation is behind it. The writer has but few sections through such regions and from only the two eyes of one specimen, so that it would not be safe to attach too much weight to this evidence until it has been corroborated from an investigation of freshly preserved eyes. If the absence of pigment in the choroid of the eye of *wagneri* is the actual condition in this wild form, it would lend added support as to the origin of the Japanese.

On the other hand, we must not lose sight of the fact, as will be shown later, that this structural peculiarity of the eye in its hereditary behavior appears to be associated with, or a manifestation of, the Japanese type of spotting, in which case it would be a mutational and not an ancestral condition.

EVIDENCE FROM PRECIPITIN TESTS.

In order to check the relationship of the Japanese waltzing mouse with *M. wagneri*, it was thought that possibly a physiological precipitin test might give some interesting results. The test for blood groups was not considered, for MacDowell and Hubbard (1922) have shown that there is an absence of isoagglutinins in the different varieties of mice and even between rats and mice. Besides, it could not have been used against *wagneri* as no blood of this form was available. The precipitin test seemed to be the logical one to try.

Since muscle proteins have proved to be fully as specific in their reactions as blood proteins, these were used for the following reasons: First, because they are more easily obtainable from such small animals, and, second, this was the only material available of the species *wagneri*, the only material obtainable of this wild form being from dried specimens. It was thought best, therefore, to simulate this condition, as far as possible in at least one test, by using dried tissue. For this purpose fresh muscle was thoroughly dried in an oven at 50° C. for 3 days, care being taken not to overheat and thus cook the meat, and it was then stored in a dry place.

The methods employed were as follows: Two groups of two rabbits each were immunized, the one to fresh mouse protein, and the other to dried rat protein. One other rabbit was used as a check. Extract of protein was prepared by taking one gram of fresh muscle and thoroughly macerating it, adding 5 c.c. normal saline solution (0.85 per cent salt) and letting it stand 6 hours at room temperature, shaking occasionally. Of the dried muscle 0.55 gms. were used, as it was found that the fresh tissue lost 0.45 gms. by weight in drying. Furthermore, in the latter case, 5.5 c.c. salt solution was used, the extra 0.5 c.c. replacing water lost by evaporation. Neither extract was filtered for injection, but the sediment was allowed to settle for a few minutes.

To immunize, inoculations were given intravenously, one a day consecutively for the first 3 days of each week, for 4 weeks. The initial amount of extract given was 0.5 c.c. which was gradually increased to a maximum of 2.5 c.c. for the sixth injection. There was no increase after this but a uniform amount of 2.5 c.c. for all remaining inoculations. These amounts seemed to be far from lethal as the animals at no time showed any ill effects and their appetite remained normal. No attempt was made to approach the lethal dosage, as Dr. Belding of the Evans Memorial Hospital, Boston, informs the writer that, in his experience, antibodies are built up with small amounts fully as readily as with sub-lethal doses. The rabbit used as a check received normal physiological salt solution at the same time as the others and in like doses.

At the beginning of the fifth week a trial test was made. The extract was tested without dilution, just as used for inoculation, except that it was filtered to render it as clear as possible. About 10 c.c. of blood was drawn from the marginal vein of the rabbit's ear and allowed to clot in a cool place. The clot was then loosened and the serum collected. With care, clear serum could be obtained in this way; if it contained corpuscles, it was centrifuged, as both serum and extract were used "crystal clear."

The following dilutions of this serum were added to the extract: 1 : 5, 1 : 10, 1 : 50, 1 : 100, 1 : 500. Each serum was tested for antibodies against its own antigen. The check was tested against both mouse and rat.

On adding the serum to the extract, no immediate precipitation was visible in any of the solutions. At the end of a half hour, a distinct cloudiness was visible in all tests, *including the check.* This result indicates that either there is no true precipitin formed, or that rabbit serum contains antibodies against mouse and rat protein.

During the sixth week, complete tests were made, using extracts of the fancy and waltzing mouse and the rat, both fresh and dried. Results were all similar to the above, and the test accordingly abandoned.

Nuttall (1904) found that one rodent can not be immunized against the serum of another to form a precipitin.

The results of this experiment show also that the rabbit can not be immunized against the proteins of the rat or mouse.

On account of the limited time, a less exhaustive test was undertaken, using a chicken and a young screech owl as sensitizers. Inoculations were intraperitoneal rather than intravenous, and were accordingly increased in amount. Injections were made 4 days a week, for 2 weeks; the owl received 0.5 c.c. and the chicken 1.0 c.c. at the start; and doses were gradually increased up to 3 c.c. and 5 c.c. for the owl and chicken respectively.

Fifteen days after the first injection and 5 days after the last, a trial test was made, using the sensitized serum against its own antigen. The following dilutions were used: 1 : 2, 1 : 4, 1 : 8, 1 : 16. With the first two dilutions precipitation was immediate. With the latter two, 3 and 10 minutes, respectively, were required to produce a visible precipitate.

Having thus tested the relative potency of the serum, complete tests were made, with results as shown in Table 3. All time reactions are, at the best, only approximate, as it is impossible to determine the exact instant at which a precipitate becomes visible.

The reactions of the several extracts to the serum of both chicken and owl are significantly different and clearly noticeable from the beginning. At the end of an hour, however, all extracts containing corresponding dilutions showed approximately equal amounts of precipitate, and were quantitatively indistinguishable. In 24 hours the precipitate had settled and showed quantitatively, in the following order: fancy, Japanese, rat and *wagneri.*

The effect of the sensitized serum of both owl and chicken on the extract of *wagneri* protein was interesting, for, while it did not react as rapidly as against its own antigen, it was appreciably in advance over its reaction on the extract of Japanese protein; and yet the total amount of precipitate after 24 hours was the least of any of the four varieties tested.

There was little or no visible difference qualitatively or quantitatively between the reaction of the fresh and the dried protein. The sensitized serum appeared to be equally potent against the same protein in either condition. On this account it is assumed that the fresh protein from *wagneri* would react the same as the dried, which would mean a significant difference between it and the protein of the fancy mouse.

These tests are not at all exhaustive, and any attempt to explain the differences in the reactions, in the absence of more data, would be purely speculative. They do, however, show conclusively: First, that there is a difference in specificity of the proteins of the three varieties of mice—fancy, Japanese and Wagner's—and of the rat (*Mus norvegicus*); and second, that the specificity of the protein of the fancy mouse is not the same as that of the Japanese waltzer, nor of *Mus wagneri.* It remains to be proven that the specificities of the Japanese and Wagner's mouse are the same. This the writer hoped to do when a sufficiently large stock of pure Japanese waltzers is available, so that these may be used as the antigen.

EVIDENCE FROM HETEROSIS.

Besides the characteristics of size, physiological tests and general appearance which indicate an origin of the two Japanese strains from *M. wagneri*, there is the matter of hybrid vigor, which, in all outcrosses of the waltzer with any other variety, manifests itself to a marked degree. This heterosis is noticeable not only in the matter of size but also in the entire body metabolism, longevity, and reproductive capacities, as will be shown later. The hybrid attains a body-size greater even than that of the larger parent, instead of intermediate, as is usual in intraspecific crosses (see Castle 1922, on rabbits). They can stand extreme adverse conditions as regards both food and water, are unusually long-lived, and show high fertility in both males and females, when bred *inter se* or outcrossed on any other variety. The degree of heterosis is almost unequalled in any intraspecific cross and is similar to that found when interspecific crosses are made. It can hardly be accounted for from long inbreeding and then outcrossing, for other instances of this do not give such hybrid vigor, as for example, Dr. Little's race of dilute-browns which have been brother-sister mated for many years and which when outcrossed produce hybrids which do not exhibit heterosis to any unusual degree.

Japanese waltzing mice interbreed freely with all varieties of the common, fancy and wild house mouse, *Mus musculus.* Von Guaita (1900) and Cuénot (1908) crossed them with several varieties; Haacke (1895) and Darbishire (1904), with the albino; Morgan (1908) with several varieties, but failed in the wild; Little (1914), with black-eyed-whites and others. Other breeders have found no difficulty in making the cross. The writer has crossed them with wild, albino, black, brown, and other varieties of domestic mice. In all cases the hybrids are perfectly fertile when bred *inter se* or with either parent or with any other variety. Sterility, when present, is due to morphological differences in size rather than to physiological incompatibility.

The writer finds no reference in the literature to any cross between the species of *Mus musculus* and *M. wagneri.* Such a cross would be extremely interesting from many points of view, not the least of which would be the check on the origin of the Japanese variety.

Sobotta (1893) states that the number of chromosomes in the waltzing mouse is the same as in the albino and wild *Mus musculus.* This, however, would not be significant as a proof of its origin, since different species of a genus may have a similar number of chromosomes.

On the other hand, since waltzing has been observed in other forms, by Cole and Steele (1922) in rabbits, by Bonhote (1912) and Hagedoorn (1922) in rats (*Mus rattus*), by Miss Lathrop (see Yerkes, 1907) in mice, and since Cuénot (1908) reports a possible spontaneous origin of a waltzing mouse in his stock of normals, the mere presence of this character alone would not be a proof of a separate origin, but would merely indicate the possibility of establishing a waltzing race from a normal wild or domestic form by a waltzing mutation.

All the evidence collected so far for the waltzing mouse indicates a derivation from *Mus wagneri*, and that it has been a household pet for generations.

THE PURE JAPANESE WALTZING MOUSE.

The pure Japanese waltzer is a small mouse, as compared with the average fancy mouse. Yerkes (1907), quoting O. Zoth, gives an adult weight of 18 grams. The Harvard strain of Japanese waltzers, which is the strain used throughout this experiment, is but slightly smaller. The average of 370 adult individuals shows a weight of 17.6 grams for the males and 16.8 grams for non-pregnant females.

All waltzers are exceedingly active, whirling, twisting, racing in a maze of activity. Under laboratory conditions, a pure waltzer has spun in a circle, almost pivoted on one hind foot, 416 revolutions without a stop or a reversal. Extracted waltzers are frequently even more active.

The males are very pugnacious and even if raised together will seldom tolerate each other in the same pen after reaching adult age. The females are none too peaceable and will frequently kill a new female placed in the pen. They are too nervous and irritable to make the best mothers, but if properly fed and not disturbed will raise about the same percentage of young as the average mouse. The litters are smaller, however, seldom exceeding 8, with an average of 4.6. By careful selection of only the best mothers, the author has established a strain of good nurses and good milkers which will raise large litters, 6 to 8, with very little or no mortality.

Besides its other characters, the pure Japanese waltzer possesses a peculiarity of the eye pigmentation. The pigment layer of the retina has its full and normal amount of melanin granules; but there is an almost entire absence of these in the choroid (Plate 4, figs. 3 and 4). This condition is continued around the eyeball even including the iris, the front layer of which is free of pigment, as can be seen in Plate 3, figure 4.

The retinal pigmentation is never sufficient to hide the blood-red choroid with its maze of capillaries. It merely tends to darken this tissue, thus giving it, in the living animal, a deep, fiery-red color, at times almost a maroon. Ordinarily the pigment in the iris conceals the interior of the eyeball and the eye appears, on gross examination, to be black. With the aid of a hand lens, however, it is seen that the black is restricted to the iris and that the pupil is deep fiery-red, the color of the inside of the eyeball. In dim light, particularly after sundown or artificial light, the pupil naturally enlarges and the iridal area diminishes. In this condition the color of the eye *in toto* is dark wine or ruby color. Rarely the sphincter muscles of the iris of one or both eyes fail to react and the pupil remains permanently distended. The eye then retains its deep wine-color in all lights, even sunlight.

This eye-color is, of course, an optical illusion, for it is a composite of a black circle with a red center. The human eye is unable to distinguish between these and the effect is a blending of the two colors.

A similar condition is found in the black-eyed-whites, where an examination of several hundred individuals showed invariably a black iris surrounding a red pupil. It is probably this condition that misled Bateson (1903) to state with reference to these mice, that the "Degree of pigmentation in the eye varies at least in one strain, some eyes being full black, others looking blackish red."

In black-eyed-white individuals the absence of choroidal pigment is probably due to the associated piebald spotting. Its inheritance, however, has not been fully analyzed as yet.

From the breeder's point of view, the pure Japanese has always been considered a feeble and delicate race and quite susceptible to disease and temperature changes. The reason, perhaps, is that in their ceaseless activity they fail to seek cover and shelter. The common mouse, if compelled to do without shelter, will usually succumb likewise. The waltzer exposes himself through restlessness, not being phlegmatic enough to stay quiet and under cover when it is necessary for his welfare. This delicacy is not due to long-continued inbreeding, for it was noted by early writers, Haacke (1895) and others. Cuénot (1908) writes "ces Souris sont en général, sinon toujours, difficiles à élever, et il en meurt un grand nombre en bas âge; il parait donc y avoir corrélation entre la valse et une santé delicate." The extracted waltzers, however, are fully as hardy as the average fancy mice and, for a generation or two at least, show the effects of hybrid vigor.

WALTZERS IN GENERAL.

On account of their almost ceaseless restlessness, all waltzers require food at frequent intervals and a relatively larger supply of water, without either of which they will die much sooner than common mice. Without food they will frequently die within 24 hours, and without water within 3 days, whereas some strains of the common mouse can, if compelled to do so, live indefinitely without water and others on a very limited amount, 15 drops per day (Keaty, Ms.).

Extracted waltzers, that is, waltzers derived by inbreeding following an outcross, are quite hardy and vigorous. Females will reproduce normally with respect to both number and size of litters. They are generally good mothers and will raise their young well. Their longevity is normal and they are not as susceptible to disease as the pure race. The males are quite vigorous and hardy. Male No. 1157, which was used extensively in the breeding for linkage, is quite a typical extracted waltzer; he is homozygous for the mutations, waltzing, non-agouti, dilution, brown, and spotting, and is heterozygous for pink-eye. In spite of the fact that he is recessive for 5 characters and heterozygous for a sixth, he is fully as vigorous as the average male mouse. He was born May 12, 1924, and the first litter sired by him was born July 28, 1924. Since then, and up to July 31, 1925, he has sired 555 recorded young, and is still a good breeder and quite active. While the majority of extracted waltzers can not claim quite such a record, due perhaps to lack of opportunity, they are far from being a feeble race. The author fully agrees with Yerkes (1907) that, although delicate in some respects, the dancing mouse is far from feeble.

Von Guaita (1900), however, reports that he was not able to raise any young from extracted waltzers bred *inter se*. Bateson (1909) and Hammerschlag (1912) seem to have had a like difficulty. The environmental conditions, especially food and housing, are probably responsible for this, rather than their genetic constitution. The writer has had no trouble at all in raising and breeding them.

Dr. Little (1924) in commenting on the delicacy of the waltzer says: "Their weakness manifests itself in a distorted Mendelian ratio which shows an excess of normal animals in crosses in which normal and waltzing are both produced." The author, however, has never gotten any distorted ratios due to prenatal or early postnatal mortality in any crosses with the waltzer, providing records were made by the tenth day. The waltzer owing to its activity uses up its energy faster and therefore does not grow as rapidly as normal young, with the result that in litters containing both normals and waltzers there is apt to be unequal growth with a resultant high mortality among the waltzers during the latter half of nursing age, at which time there is normally the highest death rate among young mice (Daniel, 1912; Gates, 1925). This differential mortality will, of course, distort ratios obtained at or after weaning, but there seems to be little or no mortality during the first half of the nursing period.

The writer finds no report as to the proportion of runts in litters of the common fancy mouse, and he has no records on this point for waltzers; yet from careful observation, it appears that the proportion of runts is no greater in the one race than in the other; and, due to the great homogeneity of pure waltzers, the proportion is probably less in this variety, than in others.

Little (1924), in a recent paper, makes the statement "It is interesting to note the variability in the character (waltzing), however, for it suggests very clearly that it is theoretically possible to obtain variations of the waltzing character extending so far in the direction of normality of behavior as to be indistinguishable either somatically or in behavior from the normal type." In this, the writer does not agree with Dr. Little. In all of the literature on the waltzing mouse which has been available, there is no mention by any of the numerous breeders of their having come across cases so nearly normal as to be indistinguishable from normal mice. In the 14 years during which the writer has bred the dancing mouse, more or less continuously, and in which many crosses have been made, he does not recall a single instance where there has been any doubt as to the classification of the waltzer. The writer grants that there are some that are more phlegmatic than others and not so nervous, active and high strung, which characteristics in the waltzer, as in other mice, are the expression of wildness; yet the peculiar behavior of the waltzer is so characteristic, and its deafness so complete and evident, with visible, tangible differences in reaction as the result of it, that there is no mistaking the waltzing character, whether it is expressed in the form of a wild maniac or a quiet, docile creature. All degrees between the phlegmatic and nervous are found in normal mice, and, the writer believes, even to a greater degree than are expressed in the waltzer. Witness Dr. Little's inbred race of dilute-browns, which are so intensely nervous that the females will seldom care for their own young!

MORPHOLOGICAL PECULIARITIES OF THE WALTZERS.

The cranial anatomy of the waltzing mouse has been carefully studied, and there seems to be a difference of opinion among the early writers as to whether there is or is not a morphological variation in the structures of the head, particularly of the nervous system, ear and semicircular canals.

Yerkes (1907), who carefully reviews the literature up to that time, states—

> All facts of behavior and physiology which have been established lead us to expect certain marked structural differences between the dancer and the common mouse. The bizarre movements, lack of equilibrational ability, and the nervous shaking of the head suggest the presence of peculiar conditions in the semicircular canals or their sense organs; and the lack of sensitiveness to sounds indicates defects in the cochlea. Yet, strange as it may seem to those who are not familiar with the difficulties of the study of the minute structure of these organs, no structural conditions have been discovered which account satisfactorily for the dancer's peculiarities of behavior. That the ear is unusual in form is highly probable, since three of the four investigators who have studied it carefully agree that it differs more or less markedly from that of the common mouse. (Page 71.)

Again (page 72) he says:

> It seems highly probable to me, in the light of my observations of the dancer, and my study of the entire literature concerning the animal, that no adequate explanation of its activities can be given in terms of the structure of the peripheral or the central nervous system or of both, but that the structure of the entire organism will have to be taken into account. The dancer's physiological characteristics, in fact, suggest multitudinous structural peculiarities. . . . That there are structural bases for the forms of behavior, which this book describes, is as certain as it could be were they definitely known.

Cuénot (1911) likewise believes that this character is due to modifications "de tout l'appareil nervo-musculaire presidant à la marche;" and further that it may even "exercé aussi son influence sur le rein, le tube digestif, le cerveau, etc."

From the experiments of Morgan (1911) in the production of artificial waltzers by the injection of acetyl-atoxyl, it would seem that the character can be simply explained by degeneration, or arrested development of nerve terminals. He says—

> I have kept artificial waltzers for more than a year, and at the end of that time, they waltzed as well as at first. It would seem probable, therefore, that the drug has caused a permanent change in the animal, and since it is known to produce degeneration of certain nerve fibers in man, it seems not unlikely that the effects on mice are of the same nature.

Hammerschlag (1912), however, who has made a very careful study of the morphology of the auditory apparatus of the waltzer, reports irregularities of varying degrees, the labyrinth and organ of Corti being most noticeably affected. He also states that the rarity with which it conforms to mathematical ratios is not to be wondered at, since it (the waltzer) is a combination of recessive characters. With reference to combining other recessive characters (pink-eye, spotting, albinism) in the waltzer, he says he has been able to obtain only one such individual and "Es war noch debiler als die Stammform und erwies sich als steril."

Similarly von Lennep (1910) finds various malformations in the auditory apparatus of the waltzer: an absence of the stria vascularis; degeneration of the organ of Corti; reduction of the cocklear nerve, and other such abnormalities.

While the writer has not made any examination of the internal structure of the skull of the waltzer, there are irregularities on the surface which suggest that there may be other malformations internally. The narrowing and lengthening of the entire skull has been mentioned. The palatine processes, which in normal mice are separated by a distance of approximately 1 mm., are sometimes so close together that their edges touch, as can be seen in figure 2, Plate 2. Other minor irregularities frequently occur, less commonly in the pure race than in the extracted forms.

SENSE OF HEARING IN THE WALTZER.

Yerkes (1907) states that in some of his young waltzers he has gotten reactions to sound between the thirteenth and the seventeenth day, but that at all other times they are deaf. All other authors, who have experimented with the waltzer, report complete deafness. The writer has tested all types of waltzers, pure and extracted, at all ages, and with all types of sound and has never had any response that could be interpreted as due to sound perception. They are very sensitive to the sense of touch and smell conveyed by air currents, or otherwise, and sometimes there seems to be a confusion as to what stimulus caused the reaction—sound or touch, or sound or smell. Whenever there has been this possibility and the mouse has shown a response, repetition of the experiment in all of its details without the sound usually shows the same reaction, showing that it is not the sound to which they reacted.

The young of the waltzer, like those of other mice, are very sensitive to all types of stimuli between the age of 15 and 30 days. It has been at this age that most tests have been made. There are two types of sounds to which mice generally respond most readily. One is a short, sharp click or psst, and the other the squeak of their own species or an artificial squeak. By quickly snapping together an ordinary pair of laboratory forceps, a sharp click is produced which will always produce a response in an ordinary mouse, no matter how familiar the sound may be; the lesser responses are in the form of a twitching of the ears. This characteristic twitching of the ears is never seen in the waltzer.

Waltzers between 15 and 30 days, together with their mothers, have been placed in a heavy earthern jar covered with a fine screen wire to prevent vibrations as much as possible, and allowed to become perfectly still, which may take a half hour or more. Then all sorts of sounds were produced close to the jar. Other young mice were even taken, held over the jar and by exciting them, were made to squeak vociferously. None of these sounds at any time have awakened the peacefully sleeping or nursing waltzers, either old or young. Similar experiments have been tried using other types of containers, such as wood, wire basket covered with cloth, etc., in each instance the idea being to prevent every possible chance of vibration, yet the result has always been negative. In most cases the mice have been allowed to become entirely quiet, i.e., sleeping; in a few, however, only partly quiet, but all results have alike been negative. Ordinary mice, of like age similarly tested, become frantic in their efforts to get away.

The waltzer has been held in the closed hand and, after it had quieted down, all types of sound have been again produced near it, and its ears carefully noted. There has never been observed characteristic twitching of the ears showing a response, as in ordinary mice.

Early in the evening when the mice are very active and there is a general rustle through all pens, if a psst sound or a pain squeak of a mouse is produced, there is no let up in the rustle made by waltzers, which continue to race around without a stop. A similar experiment with ordinary mice will cause an immediate stopping of activity until the sound has been analyzed. This experiment was tried many times in the mouse room at Cold Spring Harbor, where there were thousands of mice. Ordinary mice would cease their rustle at the first sound, begin again, stop, and then continue. Each time the response was less and less until it was not noticeable, whereas similar sounds made in the room occupied only by waltzers produced no effect whatsoever. Turning on and off of the electric lights, however, produced similar responses in both ordinary and waltzing mice, showing a response to sight on the part of both. The rustling would cease at either the turning on or the turning off of the light.

All types of waltzers have been tested, not only the pure race of Japanese, but extracted waltzers of various types, and even some that have been extracted after four generations of outcrossing. None has ever shown a response to sound. Incidentally it might be stated that a similar condition of deafness was found in a waltzing rat that occurred among the *Rattus rattus* at the Bussey Institution.

METHODS OF TESTING LINKAGE.

The method of testing for linkage was that generally recognized as being the most satisfactory, that of a back-cross between an individual heterozygous for both characters and a double recessive. However, to simplify the testing and to test as many characters as possible with one cross, using the fewest possible number of individuals, a strain possessing several readily distinguishable mutations was established. The recessive characters combined in the individuals of this strain were pink-eye, dilution, brown, piebald, and waltzing (compare fig. 1, Plate 5). Such a multiple recessive, if used in back-crosses with animals heterozygous for all these characters, would give at once a linkage test for 10 pairs of factors, namely, waltzing with pink-eye, dilution, brown, and piebald; piebald with pink-eye, dilution, and brown; brown with pink-eye and dilution; and dilution with pink-eye. Actually, however, for reasons stated below, most of the data were obtained in a back-cross to a four-point recessive, rather than a five-point.

The tests for agouti linkage were made by back-crossing F_1 to a triple recessive, and those for albinism by back-crossing F_1 to a double recessive; while those for short-ear and kinky-tail are a straight F_2 generation. The agouti factor was eliminated from the four and five point cross, because of the difficulty of distinguishing between agouti and non-agouti in the pink-eyed dilute forms. Albinism was also eliminated on account of its epistatic nature, which completely conceals all of those characters which can only express themselves in the presence of color. In back-crosses involving albinism, 50 per cent of the individuals are worthless for classification, as they are albinos, the genetic constitution of which can only be determined by breeding.

To obtain the five-point recessive, the pure Japanese waltzer, which is a piebald, was crossed with a pink-eyed dilute-brown. The Japanese thus introduced, into the hybrid heterozygote, two recessives (waltzing and piebald) and three dominants (the allelomorphs of pink-eye, dilution, and brown respectively). The other parent introduced the five contrasted characters. This cross produced individuals having all the characters of the wild mouse except the gray (agouti) coat, instead of which they had a black coat.

ASSOCIATION SYSTEMS OF MENDELIAN FACTORS.

The Japanese waltzing mice used in this cross were of the Harvard strain, raised by Mrs. Lambert of Boston. These originated, if the author is correctly informed, from a single pair obtained from Professor Yerkes about 1909. They have been inbred, but not always by systematic brother-sister matings, since that time. The author's present stock was obtained from Mrs. Lambert in the spring of 1920 and has been carefully brother-sister mated, as far as possible, since that time, exceptions being made only on the absence or death of males in a litter, and once due to the loss of breeding females. This stock is, therefore, extremely homogeneous and for that reason forms admirable genetic material. It is this stock which has been used throughout the experiment.

The use of this waltzing stock, and of an inbred stock of dilute-brown mice as the other parent race, afforded an excellent opportunity to test the hypothesis of association of groups of interspecific characters aside from linkage. Cook (1909) in working with cotton crosses finds that all the characters of each parent apparently form a group which does not readily segregate and recombine in expected mathematical ratios. He says—

> In hybrids between different groups of cotton, there is a distinct and very general tendency to coordination or coherence of characters . . . (likewise) closely associated characters tend to preserve their coherence . . . Incongruities are rare and have only been found thus far on plants which are nearly sterile or otherwise definitely degenerate. . . . In Mendelian hybrids the characters derived from the same parent show no such coherence of expression, but appear in all sorts of combinations with characters of the other parent.

This hypothesis is corroborated by Jesenko (1913) who reports alternative inheritance of character groups in wheat-rye crosses. Goodspeed and Clausen (1917) obtain clear association of characters in interspecific tobacco hybrids, in which the entire group of parental characters tend to cohere, the viability of the individual depending on the association of such "contrast systems." Furthermore these authors express the view that "Strictly Mendelian results are to be expected only when differences are within a common Mendelian reaction system." Such a condition in animals was also suggested by Nabours (1912) and by Cook (1913) who accounts for Nabours's results in the cross *Bos indicus* with *B. taurus* on this basis. Nabours states "It is clear that in the F_2 generation, pure Brahma and pure Durham are segregated."

In the case of the waltzing mouse, there have been associated throughout its breeding history—and there are reasons for believing with Yerkes (1907) that the race has "existed for several centuries,"—four prominent characters, namely, pigmented eye, black-coat color, spotting, and waltzing. All of the earliest reports (Brehm, 1890; Haacke, 1895, and others) describe the Japanese waltzer as a black-and-white spotted mouse. Yerkes (1907) says that although he obtained animals from various dealers in different places they were "all strikingly alike in markings, size, and behavior," and that they "were white with patches, streaks or spots of black." Darbishire (1902) and his associates, however, used a pink-eyed spotted form, which, from the fact that all hybrids produced by outcrossing possessed a black coat-color (black agouti or black), must have been a potential black. Yerkes (1907) states that "all of his animals were black eyed," which probably indicates a "continental" or Asiatic rather than an English origin. Since the author's stock is descended directly from Professor Yerkes's mice, it is safe to assume that the three characters mentioned above have been associated in the waltzer throughout many generations of inheritance and years of breeding.

From what has been said relative to the origin of the pure waltzer, and from the fact that the characters, aside from waltzing, which it possesses today have been associated with it since very early times, it is fairly certain that these characters are mutations of the original wild *Mus wagneri* rather than introduced through hybridization. This explanation probably accounts for the peculiar type of spotting found in those races originating from the *wagneri*, a type which, as can be seen from the photograph, differs from the other, or black-eyed-white type. If then there is anything homologous in animals to the association of groups of characters as in cotton and tobacco hybrids, we should have an indication of such a "contrast system" in this cross. As will be shown later, this is apparently the case.

It is also of extreme interest to note that all of the *surviving* strains of waltzing mice are black and white spotted. Nearly all of the earlier experimenters crossed the waltzer with all types of fancy mice and produced waltzers in all of the prevalent color combinations, yet *none* of these seems to have survived to establish a strain.

Two other characters, dilution and brown, likewise, have been associated together since the origin of the other parent variety, through many years and generations of consistent and close inbreeding.

On account of these facts, and also that the two species interbreed freely, it probably would have been impossible to have selected any type of mammal material which would have afforded a better opportunity for testing this hypothesis of the association of parental "systems" of characters.

THE PINK-EYED, DILUTE-BROWN STRAIN.

The pink-eyed, dilute-brown strain, which forms the other parental stock, was derived from an initial cross of a pink-eyed, dilute-brown male received from Dr. L. C. Strong, which in turn was derived from an outcross of Dr. Little's inbred, dilute-brown to a pink-eyed stock. This first male was bred to several dilute-brown females (Little's stock), and pink-eyed, dilute-brown individuals were obtained in the F_2 litters, and from the back-cross of the F_1 females with the original male. In this original cross, the expected Mendelian ratios resulting both from *inter se* matings and from back-crosses were approached, although the numbers are quite small. From the *inter se* matings with an expected ratio of 3 : 1, 53 individuals were obtained, of which 35 were dark-eyed and 18 pink-eyed. The back-cross, which should yield a ratio of 1 : 1, gave 6 dark-eyed and 7 pink-eyed individuals, in excellent agreement with expectation.

This pink-eyed, dilute-brown race was then carefully tested by inbreeding for two generations and showed itself pure except that some individuals were heterozygous for albinism. With this stock pure, except for albinism, the first set of crosses was made. Albinism was finally eliminated and the purified pink-eyed, dilute-brown stock was inbred for 7 generations before again being crossed with the waltzer to produce the experimental F_1 generation.

THE HYBRID.

While the following description deals primarily with the cross between the waltzer and the pink-eyed, dilute-brown strain (fig. 2, Plate 5), it will apply in its general principles to any cross involving the former as one of the parents.

In the cross under discussion, all single-gene characters (except self) show, as would be expected, complete dominance. The F_1 animal is a normal, black mouse, but incomplete dominance of the self-factor shows in the white feet, patches on the tail, and occasionally in a white spot, or even a large patch along the median line of the belly (Plate 5, fig. 2).

The first series of crosses with the waltzer was made with the plan in mind of breeding the F_1's *inter se*, so as to extract an individual with the five recessive factors that entered the cross, namely, pink-eye, dilution, brown, piebald, and waltzing. According to expectation, when F_1's heterozygous for five Mendelian characters are bred *inter se*, an ultimate recessive homozygote for all 5 factors should occur once among 1,024 individuals. Actually the first ultimate five-point recessive to occur in the F_2 generation was the 691st individual to be recorded, and out of a total population of 1,146 recorded individuals this was the only complete recessive obtained. It was hoped that by obtaining a few individuals of this type, linkage could be readily tested by back-crossing F_1 individuals, heterozygous for all 5 factors, to such a homozygote.

Actually it was found far more convenient to use a four-point recessive, because the young could be identified earlier. This would release the mother and she could be returned to the breeding pen, all of which would permit of producing greater numbers. The testing was, therefore, done by back-crossing to individuals homozygous for 4 recessive factors. The most satisfactory combination proved to be a dark-eyed, dilute-brown, piebald waltzer (Plate 5, fig. 1) that was heterozygous for pink-eye. This would give a rather unsatisfactory test for the relation of pink-eye to waltzing. But since pink-eye and albinism lie in the same chromosome, and since the latter and waltzing have been tested independently of the former, we may say that what is true of one is probably also true of the other as regards the occurrence or non-occurrence of linkage.

The first generation hybrids from this cross were exceedingly vigorous and long-lived. hybrid vigor Heterosis is quite apparent in their larger size, which is slightly greater than that of the pink-eyed, dilute-brown parent. The average weights, however, are about equal, for in 50 non-pregnant, adult, female hybrids it was found to be 24.8 grams, while for an equal number of pink-eyed, dilute-brown females it was 24.6 grams. Both sets of individuals were selected at random after being separated from males for 20 days. The average weight of the Japanese male, as stated above, is 17.6 grams. Intraspecific crosses involving two such individuals, would, in general, aside from heterosis, give an animal of intermediate size. In the matter of size, therefore, the outcross exhibits its hybrid vigor. In other respects, however, where hybrid vigor is not concerned, we find the normally expected "blending" of characters. As for example, the average tail-length of the hybrid is 76.2 mm., while those of the parents are 60.0 mm. and 87.1 mm. respectively; the average number of scale rings of the tail of the hybrid is 170.0, while that of the two parents is 141.0 and 180.8 respectively. Likewise the number of tail vertebræ in the hybrid varies between 23 and 25 inclusive, while those of the parents are 21 to 22, and 26 to 28 respectively. In the matter of litter-size the hybrid also is intermediate. The average litter-size of the Japanese is 4.6 ± 0.14 and of the pink-eyed, dilute-brown 6.1 ± 0.15, while that of the hybrid is 5.7 ± 0.09.

MEASUREMENTS.

A study of the body-measurements of the hybrid (Table 2) reveals interesting conditions. The actual body-length is a little less than that of the common fancy mouse, but the weight is about the same. The dimensions of the skull are larger throughout (Plate 6, fig. 2). The average cranial length is 23.8 mm. as compared to 22.00 mm. for the fancy mouse. This difference becomes quite significant when we consider that the largest among 111 skulls, of both males and females of the fancy mouse, measured 23.7 mm., a fraction less than the *average* of the hybrid skulls. The latter range in length from 23.3 mm. to 24.4 mm. Other skull measurements are given in Table 2.

Not only in size is the hybrid skull larger, but in weight also; it averages 0.26 grams per skull, as compared to 0.22 grams of the fancy. Both sets of skulls were similarly cleaned and thoroughly dried before weighing.

From the above it is obvious that the hybrid between the Japanese male and the pink-eyed, dilute-brown female is of bigger frame than even the larger of its two parents. Measurements of the latter given in Table 2 show it to be a normal fancy mouse, quite similar to any of the numerous other varieties. On account of its less phlegmatic disposition and greater reproductive activities, however, the cross-bred animal does not as readily take on fat. The writer has never had any which showed a tendency to accumulate fat and become sterile, a condition frequently found in other mice, especially after casting four or five litters.

METABOLISM.

Repeated tests had shown that neither the waltzer nor the pink-eyed, dilute-brown animals can live without water, yet the hybrid has been kept for 4 months on an essentially dry diet of commercial horse feed, containing cracked corn, oats, and alfalfa sweetened with a little molasses. Chemical analysis of this feed showed a maximum of 16 per cent total moisture. Commercial dog biscuit was also fed, which gave a chemical analysis of 7 per cent total moisture. On this dry diet the hybrid mice did well, their weight after the first few days remained normal and uniform, and they bred normally. They were, however, unable to raise more than one or two young per litter as the secretion of the mammary glands seemed to be greatly lessened on the dry diet. In all other respects their metabolic and reproductive capacities did not seem to be affected by the absence of water other than that contained in the food.

LONGEVITY.

The longevity of these hybrids is unusual. Several of the original F_1 individuals are, at the time of this writing, still alive, active and reproducing at an age of over 24 months (Plate 6, fig. 1). Only a few of the original hybrids have been recorded as killed on account of old age. In not a single instance has one of these hybrids proved to be sterile even up to an advanced age. Yerkes (1907) mentions having had a waltzer that lived 2 years, and the writer had a Japanese male that lived to be 28 months old, but these are exceptional cases and nearly always the individuals are sterile after a year. The one male mentioned above was fertile up to an age of 20 months, a very exceptional record.

REPRODUCTION.

It is a generally accepted view among breeders of mice that the reproductive life usually ends when the mouse is about a year old. During this time the female will, on the average, have raised 4 to 5 litters. Of course exceptional individual cases occur where the female has produced more and lived longer, but these are unusual. This is the view expressed by Daniel (1912) who states that sexual maturity begins at about 2 to 3 months and at the "end of 10 to 12 months of age activity diminishes, and for purposes of breeding the mouse is of little further service." Kirkham (1920) on the other hand, reports an age of 10 to 22 months for the white mouse with a maximum of 12 to 16 litters and 80 young. The writer is inclined to believe that Kirkham's mice must have been exceptional and kept under very favorable conditions, and that the females did not nurse their own young. Of course much depends on whether or not the mouse nurses her own young and raises them. More litters and more young can be gotten from any female if the young are removed at birth and she is returned to the breeding pen.

Even in rats, which are naturally longer lived than mice, King (1924) reports that while the length of the reproductive period varies considerably in different strains, it, as a rule, covers from 12 to 15 months; the youngest and oldest limits being 56 days in the albino to 33 months in a pigmented individual. She says—

> The females begin breeding when from 3 to 5 months of age and reproductive activity extends over a period of from 12 to 15 months, regardless of the coat-color of the individual. Although females may cast as many as 14 litters, as a rule they produce only 4 or 5 litters during the course of reproductive life.

Donaldson (1924) likewise states that in rats "Menopause occurs at 15 to 18 months."

Contrast this with the reproductive life of the F_1 hybrid between the waltzer and the pink-eyed, dilute-brown. Of the first cross, born in July, August and September, 1923, 39 females were raised. These produced from January 1, 1924, to September 15, 1924, 1,309 young, all litters of which were raised at least to an age of 6 to 8 days. About September 25, these mice were shipped to Baton Rouge, Louisiana, where it was thought hardly worth while to keep them as breeders any longer on account of their age, 12 to 14 months, and most of the breeding pens were isolated and some of the mice used for other experiments. It was these old (?) mice that Miss Keaty used to test their ability to live without water. It is truly remarkable that animals of this age were able to maintain their normal metabolic and reproductive capacities at such an age and under such adverse conditions. The writer then began to realize the extraordinary hybrid vigor of these animals. The survivors, for several had been killed for various experimental purposes, were gathered together and shipped back to Boston when the author returned to the Bussey Institution. Here they were remated and used for linkage testing. Some of these have had to be killed on account of old age and bodily emaciation. Several, however, are still active, in good health and reproducing at the time of this writing, August 1, 1925, in spite of their 24 months of age, and the indications are that they will still be in condition to ship back to Baton Rouge at the end of the present summer.

Female No. 583 is quite typical and probably represents about the average. She certainly is not an extraordinary hybrid female. She was born on July 18, 1923, has traveled three times between Baton Rouge, Louisiana, and Boston, Massachusetts. She was photographed on April 19, 1925, age 21 months, just before the birth of her thirteenth litter. This litter, cast on April 22, was composed of 7 young, 4 females and 3 males. Up to this date, this hybrid had given birth to 71 young in 13 litters. Her fourteenth litter consisted of 4 young, making a total of 75 young. She has now been isolated to test her longevity. Of this number the records show but one young which died before its characters were recognizable. There is no record of any of the young of No. 583 being transferred to foster mothers, which means that she raised all of her young, with but one mortality, to at least an age of 6 or 8 days. In at least one instance, the records show that she raised two of her young, saved for breeding purposes, to weaning age.

Such vigor and reproductive capacity as this is exceedingly rare, if not entirely absent, in any of the varieties of ordinary mice. It certainly is not found in either of the two parental races; it is probably due, as stated before, to hybrid vigor produced by an interspecific cross.

The females are exceptionally good mothers and under favorable conditions will raise practically all of their young. Depletion of litters has been through abnormals (runts, etc.) and pathological individuals and not through carelessness or other acts of the parent. Eating of the young does not occur, so far as known.

CARE AND BREEDING.

All of the mice used were kept in wooden boxes or wire cages. The boxes seemed preferable to keep pregnant females in and for raising the young. In the wire cages the young seemed to be more prone to scatter and get cold. Occasionally they would fall between the meshes of the wire, so, as far as possible, all pregnant females were placed in wooden boxes which gave a minimal loss of young, and as consequence resulted in few depleted litters. This is of considerable importance in dealing with factors which may tend to produce differential mortality among the young and a consequent distortion of results.

Aside from the sex-linked lethal reported by Little (1920) in the waltzing mice, there is nothing in the literature to indicate a prenatal mortality in the strains of mice used, similar to that found in the yellow and possibly in black-eyed-white mice. And since the size of litters and the sex-ratio in all generations have been more or less normal, if we may judge by comparing the average litter-size of the common mouse, 7.4 (Gates 1925), with that of the Japanese waltzer, 4.6, it has been assumed that no prenatal mortality exists which would affect the total result. This possible source of error may, therefore, be disregarded.

All pens of mice were carefully gone over every 2 or, at the most, 3 days. The pregnant females were separated at this time and placed in clean nesting compartments in wooden boxes, the nest proper being separated from the runway by a wire partition, the two connecting by a small opening. The nesting material used was shredded paper. The precaution of Detlefsen and Roberts (1918), of having perfectly clean nesting material, was followed, to avoid any possible chance of the mother destroying any young without leaving some visible evidence.

Pregnant females were examined daily, and at the birth of a litter the young were recorded immediately as to number and sex. The mother was then left unmolested for 5 or 6 days, at which time the young were examined and classified. On the seventh or eighth day the young were again examined and the accuracy of the previous data checked, both with respect to sex and characters involved. Usually all the characters concerned in this cross are readily distinguishable at an age of 5 to 6 days, but for the sake of accuracy all young were rechecked 2 days later. At this time the young were killed and the mother returned to the breeding pen. Rarely, in the case of pink-eyed, dilute individuals, and always in the case of dwarfs or pathological individuals which failed to grow normally, the affected individual was transferred to a foster mother to give it a chance to develop up to the point where its characters could be determined. Sometimes also for the sake of economy of time, foster mothers were used and the entire litter transferred to them as soon as born. All such foster mothers employed were either albinos or agouti mice so that there would be no possible chance of any error through failure to remove all young borne by the foster mother.

THE F_2 GENERATION.

The symbols used to denote the various factors involved in this experiment are those generally employed by geneticists and follow on the whole the suggestion of Castle (1913) of denoting the character by the mutation rather than the normal. They are A = agouti, b = brown, c = albinism, d = dilution, k = kinky-tail, p = pink-eye, s = spotting, se = short-ear, and w = waltzing. The normal allelomorph of each of these is denoted by the corresponding capital or small letter. Thus ABCDKPSSEW would represent the normal wild mouse as far as the characters dealt with in this experiment are concerned; while abcdkpssew would be an albino which would also be genetically a non-agouti, brown, dilute, pink-eyed, piebald, short-eared, kinky-tailed waltzer.

The straight F_2 generation, that is, individuals gotten by brother-sister matings of F_1 individuals, gave some very interesting results. Yet such a mating, involving five Mendelian pairs of factors, is far from satisfactory for determining linkage of characters, primarily for the reason that the mathematical ratio involved is too complicated. It is 243 : 81 : 81 : 81 :

81 : 81 : 27 : 27 : 27 : 27 : 27 : 27 : 27 : 27 : 27 : 27 : 9 : 9 : 9 : 9 : 9 : 9 : 9 : 9 : 9 : 9 : 3 : 3 : 3 : 3 : 3 : 1. This means that theoretically there should be 243 individuals that would show all 5 of the dominant characters; 81 of each of 5 groups that would show 4 dominants and 1 recessive; 27 of each of 10 groups showing 3 dominants and 2 recessives; 9 of each of 10 groups showing 2 dominants and 3 recessives; 3 of each of 5 groups showing one dominant and 4 recessives; and 1 ultimate recessive showing none of the 5 dominant characters. Furthermore, in the first group there are 23 combinations by which parental gametes may unite to produce an individual that will show all 5 dominant characters; 41 different combinations to produce each of the 5 groups of 4 dominants; 14 different combinations to produce each of the 10 groups of 3 dominants; 5 different combinations to produce each of the 10 groups of 2 dominants; 2 different combinations to produce each of the 5 groups containing only 1 dominant; and only 1 combination that will produce the 1 ultimate recessive.

Of course it is only necessary, when tabulating results for linkage, to consider 2 characters at a time, but with the almost endless number of combinations possible, there is a great chance of modifying the results by the variety of factors which enter the combination but which are not considered in the particular pair under observation. As for example, when tabulating data on the possible linkage or non-linkage of piebald and waltzing, there may be the modifying influences due to the three other factors in all possible combinations and in homozygous or heterozygous condition.

It is mainly on this account that the straight F_2 generation is not considered in the linkage tests. Yet, as stated above, this mating gave some interesting results. It was found for instance that there was a tendency for the entire group of characters of each parent to associate together. Thus we have 61 individuals out of 1,146 having identically the same combination of characters as the male parent, i.e. the waltzer, when according to Mendelian expectation we would expect but 30, a difference of 31, which is 5.27 times its probable error, ± 5.88. That such a condition is not a mere accident is shown when we tabulate the parental characters in smaller groups of 4 and 3 each (see Table 4).

As seen in this table, there is a persistent tendency in every possible combination, but one, for the original characters to appear in association in greater than chance frequencies. The excess numbers obtained, over and above expectation, vary from 5 per cent in the dense, black, waltzing group to 77 per cent in the dark-eyed, dense, piebald, waltzing combination. Expressed in other terms, the former is 1.6 times and the latter 5.6 times the probable error. The other groups vary between these two limits. The only one that shows a deficiency is the dense, black piebald combination, and here the difference is but one. This deficiency is accounted for by the paucity of the pink-eyed individuals of this group, which are considerably below expectation; omitting these, there would be an excess, as in the other groups. It seems hardly possible that random sampling could account for such consistent results. This condition will be taken up again in considering the back-cross where similar results are found.

Aside from the above-mentioned association systems, the F_2 generation showed no apparent grouping of pairs of factors which would indicate linkage. All the visible characters seem to show independent assortment and segregation except for the parental grouping.

THE BACK-CROSS GENERATION.

ASSOCIATION SYSTEMS.

As stated above, the test for possible linkage of waltzing with pink-eye, dilution, brown and spotting was made by back-crossing males, heterozygous for pink-eye but homozygous for the other 4 factors, with F_1 females heterozygous for all 5 factors. The males were selected from the straight F_2 generation, and the females were obtained by crossing a spotted waltzer (pure Japanese male) with a pink-eyed, dilute-brown female (compare Plate 5, fig. 2).

This back-cross mating produced offspring of 32 different somatic types, all of which should occur in equal numbers except the pink-eyed classes and these should be one-third as numerous. All other tests were made by using in each case a simple dihybrid combination, either back-cross or F_2, producing but four types of individuals.

Up to date, 1,289 individuals have been recorded in the first cross, 504 in the albino cross, and 111 in the agouti cross. These figures include individuals from *undepleted* litters *only*. All litters in which one or more young died before being classified are rejected. It is interesting to note in passing, however, that in the discarded litters there is no indication of differential mortality, at least up to the age at which the young were recorded, Mendelian ratios being normal, despite the small numbers. The most careful attention was given to detecting depletion in litters by daily observation and recording of all litters born. Yet, in spite of this, the number of depleted litters is practically negligible, being less than 3 per cent.

As previously stated, judging from litter-size and sex-ratios (1,177 females to 1,167 males), there is nothing to indicate any differential prenatal mortality and, to avoid any possible source of error due to postnatal deaths, only complete litters are used in tabulating results. Thus, the precarious life of the waltzer, in its competition with the normal is not discriminated against in the evidence.

Table 5 gives the detailed record of all classes and the number of individuals of each class expected and obtained.

That all characters are behaving normally from a Mendelian standpoint is indicated by the ratio between the expected and the actual number of recessives (see Table 6). These are in close agreement with the hypothetical with the exception of non-agouti and albinism. The former shows a deviation of 4.64 times the probable error (Table 6) and is most likely due to the small numbers involved. As seen in Table 4 the deficiency occurs in the two classes: non-agouti, spotted normal; and non-agouti, self-colored waltzing. A paucity in these two classes could hardly have any significance and is probably the result of random sampling.

The excess of albinos, amounting to 4.62 times the probable error (Table 6), is possibly significant and is not so easily explained, since it occurs in all types of matings. Reciprocal crosses show approximately the same deviation from expectancy. The reports of other investigators, Durham (1908), likewise, show an excess of albino individuals in out-crosses. No explanation has been offered, and the writer does not venture to make any in the absence of further data.

All the other mutations involved show normal distribution according to Mendelian laws (Table 6). Waltzing tends to appear in slightly excess numbers at times. This deviation, however, is due largely to the association of parental characters of the Japanese waltzer. If the one class of offspring having the characters of the Japanese be disregarded in the tabulation, the percentage of waltzing individuals would be close to the theoretical. Likewise the data from extracted waltzer crosses, where the parental association has been destroyed, show that this factor behaves normally in its inheritance. Taking into consideration this association, all deviations of waltzing easily come within the range of random sampling and the total population involved is sufficiently large to warrant a fair degree of accuracy.

With all allelomorphic factors behaving normally, we would naturally expect other Mendelian ratios to be normal, unless distorted by linkage or lethal factors, neither one of which is in evidence. However, some remarkable deviations from expectation as regards the recombination of characters have been observed.

Nothing in the results obtained indicates linkage of any two factors involved, therefore we are led to conclude that each of the genes tested is located in a different chromosome. This would mean free segregation of each character with subsequent random recombination according to the laws of chance. With 5 pairs of factors entering the cross, chance recombinations would give 32 different types, occurring in definite mathematical ratios. But, on the contrary, there appears to be a definite tendency on the part of the chromosomes to associate together according to the parental grouping, thus distorting the theoretical proportions.

This fact was noted in discussing the F_2 generation, where the tendency to associate distorted the expected results to the extent of 5.27 times the probable error. In the back-cross, the same condition exists. If all factors considered in this cross were independent and belonged to a common Mendelian system, there would be free segregation and chance recombination. This would mean that theoretically there would be 58.8 individuals of each of the 16 dark-eyed classes and 21.7 of each of the 16 pink-eyed classes. However, of the dark-eyed, dense, black, piebald, waltzing class, the combination of the Japanese parent, there were actually seen 86 individuals (Table 5). This is an excess of 27.2 or 31 per cent over and above expectation, which in a 3 : 1 ratio is 6.27 times its probable error of 4.20. The deviation is therefore quite significant. These figures are based on a total population of 2,435 individuals, 1,146 F_2 and 1,289 back-cross. The closeness of the results in the two series would lead us to believe that there is a very definite tendency equally operative in the two series.

The chromosomes of the female parent, as representative of the fancy mouse, do not seem to group together as strongly as those of the Japanese parent, unless we can assign the excess of all pink-eyed classes to this cause, in which case the tendency is about equal. If the grouping is not as definite it is probably due to its more frequent out-crossing and less homogeneous nature. This latter is probably the correct interpretation.

As in the case of the F_2 generation, there is the widest divergence from expectancy in that class containing the greatest number of parental characters in combination. The numbers come closer to the hypothetical chance ratios, the smaller the number of factors considered, as shown in Table 4. This is most natural, for with the elimination of one or more factors, each group would include one or more classes in the recombinations and would have a tendency in the opposite direction. Thus in groups of only 3 characters, there may be classes in which 3 out of 5 factors, being of one parental group, would have a positive tendency; while the other 2, being of the contrasted system, would tend to neutralize the effects of the first.

That this condition is not due to abnormal random sampling is shown by the fact that in both the F_2 and back-cross matings there has been an excess of the parental groups at *all* stages of the experiment. Furthermore, if the two parental classes are eliminated, and only the remaining 30 classes considered, all Mendelian ratios approximate expectation.

These association systems appear to be specific only, and do not apply to intraspecies crosses where the chromosomes form a common Mendelian system, homologous in the different varieties of the species. In the former, there is grouping of chromosomes; in the latter, free assortment. This is clearly shown in other crosses of this experiment where extracted waltzers were used in the place of the pure Japanese. Such is the case, for instance in the tests for agouti, albinism and short-ear; in these there is no excess of the parental class, but rather a close approximation to expectancy.

To test this grouping further, a study of body and skull measurements was made to see if these characters tended to group with the more obvious factors according to the parental association. Six individuals, each of the parental type, were selected at random at an age of 5 days and raised to maturity. These were kept under identical conditions, in some instances raised together as litter mates. They were killed and measurements taken at an age of 60 days, so as to obviate any differences in age. The average measurements of these are given in Table 2 under the headings extracted PDBsw and extracted pdbSW.

Here again there is evidence that specific size-factors tend to associate or group together along with other characters of the species. The differences between the two types is not large, and if taken individually would not be considered significant. But *all* measurements show a consistent trend, each toward its own parental type. This fact becomes still more significant when they are compared to the average of miscellaneous waltzers of similar age picked at random from a mixed population. The average measurements of 6 types (12 individuals) of waltzers are given in the last column of Table 2. Observe that in nearly every case the extracted parental forms tend to diverge from the average of the miscellaneous waltzers, each toward its own specific type.

It is interesting also to note this tendency in the cranial peculiarities. Thus, there is a lengthening of the interdental gap and a slight narrowing of the palatine bridge in the derived Japanese class; both features being characteristic of the pure race. The opposite is true likewise of the contrasted type. The number of tail vertebræ varies significantly. There is a range of 24 to 26 in the Japanese class; 25 to 28 in the pink-eyed, dilute-brown class; and 23 to 28 in the miscellaneous class.

As would be expected in the offspring of hybrids, there is a greater range of variation in the derived forms than is found in either of the pure races, and it is possible that the deviations here found might be the result of random sampling, in view of the small numbers involved. Yet, on the other hand, the consistency with which they vary in definite directions seems to indicate a rather positive trend.

This condition is all the more surprising considering the fact that association is *not* complete, so that when several other characters, each in itself the result of multiple factors, are added to the original number, the chances of obtaining a complete grouping are very small indeed. Furthermore, all body and skull measurements are probably influenced more or less by heterosis, which tends to conceal certain genetic characters, as shown so positively in the F_1 generation. Hybrid vigor is apparently continued in this particular cross, into the F_2 generation at least, and probably further, if we may judge by the greater vitality, resistance to disease and temperature changes of the extracted waltzers.

LINKAGE RELATIONSHIPS.

As to linkage relationships, it might be said in general that none of the characters tested shows linkage with any other. Exception might be made, however, to the two characters incidentally involved, namely, short-ear and kinky-tail, where linkage is apparent.

While the main purpose of the experiment was to test the possible linkage of waltzing with the other characters of mice, yet the other characters have been involved in dihybrid combinations with each other in such a way as to give linkage tests between them. It is interesting to note that all of these are confirmatory of the results of other workers, in so far as the factors have been tested (see Table 7).

Table 7 gives the tabulated results of the tests for linkage. Each dihybrid combination is shown together with the expected and the observed numbers of the parental combinations and the recombinations. Some of these figures might easily be interpreted as signifying linkage, but in every instance where such is the case, if the parental classes are disregarded, the results will be in close agreement to the expected numbers. Note that this distortion does not occur in those crosses where extracted waltzers are used in the place of the pure race (albino, agouti, short-ear, and kinky-tail). All aberrant results are directly traceable to the chromosomal association of the species. For example, in the dihybrid combination of dilution and brown, the parental classes show an excess of 57.5 which is 4.76 times the probable error (Table 7) and would ordinarily be interpreted as indicating slight linkage. However, eliminating the two parental classes, on account of their distorting effects, and tabulating the results from the remaining 30 classes, there is a deviation of only 10.3 from expectancy, which is but 0.89 times its probable error. A closer agreement to the hypothetical could hardly be expected. A like condition holds true of other combinations and may be similarly interpreted.

The possible association of genes for dilution and spotting and for brown and spotting have not been reported except in small numbers. In this experiment they appear to be independent and show free segregation. The number of individuals involved is sufficiently large to make the results significant. Here again allowance must be made for the excess in the parental classes due to chromosomal association.

Waltzing appears to be independent and to show free segregation and random recombination with respect to agouti, albinism, pink-eye, dilution, brown, Dutch type of spotting, short-ear, and kinky-tail. The data indicating this is tabulated in Table 7. The numbers involved, in most cases, are large enough to be significant. The smaller numbers, however, are in full accord with the Mendelian expectation, as far as they go.

The test against pink-eye involves an unusual cross in that both parents were heterozygous for pink-eye, but one was homozygous and the other heterozygous for waltzing. This means that for linkage tests only the 2 pink-eyed classes can be considered; the pink-eyed normals representing the parental combination and the pink-eyed waltzers the cross-over or recombination class. These occur in approximately equal numbers, according to expectancy and therefore show independent segregation and no linkage. This cross, however, would hardly be conclusive by itself, but a further test of the relation of these two factors is afforded by indirect evidence gained through the test against albinism. Albinism and pink-eye are known to be linked, and therefore any factor which is independent of the one should also show free assortment with respect to the other. Albinism and waltzing segregate according to Mendelian expectancy, both in the F_2 generation and in the back-cross, from which we would expect pink-eye and waltzing to react similarly, and this is in harmony with observation (see Table 7).

The numbers involved in the test between waltzing and agouti are small and can hardly be considered conclusive; yet, as far as they go, the ratios are normal and it is probable that these two genes are independent.

With respect to the possible linkage of short-ear and kinky-tail with waltzing, the figures are not conclusive, yet quite significant in their normality, as far as they go. These tests were made by using the ratio of a straight F_2 generation. The cross was made by using extracted waltzers and thus eliminating the distorting effects of the pure Japanese. The influence of the pure race is quite noticeable by its absence. There is random recombination of both short-ear and kinky-tail with respect to waltzing, from which we conclude that these factors are independent of waltzing.

The kinky-tail character appearing in the short-eared stock is quite definitely linked with the factor short-ear. The numbers involved are large enough to warrant conclusions. There is a deviation of 86 from expectation which is 15.01 times the probable error, and is therefore quite significant. The cross-over percentage can not be determined from an F_2 generation, yet the large excess of short-eared, kinky-tailed individuals in the double recessive class (Table 5) would indicate that the percentage is probably quite small.

EYE PIGMENTATION.

As previously stated, the Japanese waltzing mouse possesses a characteristic pigmentation of the eye, in that only the pigment layer of the retina contains pigment, where normally both this layer and the choroid are colored. Whether this is due to an independent factor which inhibits production of color in the choroid, or whether it is merely a mode of expression of the Dutch spotting, the writer is not prepared to say. Indications are that it is the latter. If the former, it is apparently completely linked with this type of spotting.

Although no comprehensive attempt was made to analyze the genetics of this character, yet its behavior in inheritance was noted in many of the classes of individuals involved in this experiment. The analysis of this condition is impossible in the pink-eyed forms on account of the paucity of pigment in the eye, therefore only dark-eyed individuals were examined.

Eyes for examination were fixed in Bouin's solution, sectioned in paraffine, and lightly stained in Eosin.

In the pink-eyed, dilute-brown race there must be a potentiality, probably the self-factor, which is dominant, for the production of pigment in both the retina and choroid, for when crossed with the Japanese the F_1 hybrids always have black eyes with both retinal and choroidal layers pigmented, as shown in Plate 4, figure 3. The other condition, that is, pigment in the retina only, found in the pure Japanese, is seen in Plate 4, figure 4.

Every class of dark-eyed individuals obtained in the back-cross of the hybrid to a homozygous recessive was examined. This included the character of both spotting and self in every possible combination with black and brown, density and dilution, normal and waltzing, making 16 classes.

In all cases, the Dutch spotted individuals, of whatever other constitution, showed retinal pigmentation only; while on the contrary, every self individual, irrespective of whatever other factor it was associated with, showed both retinal and choroidal pigmentation; the quantity and kind of pigment depending on the factors for density or dilution, and black or brown. Miscellaneous individuals from other crosses, involving agouti, spotting, and waltzing were also examined, and similar conditions found. All spotted forms, whether agouti or non-agouti, normal or waltzing, black or brown, dense or dilute, had no choroidal pigmentation, while all selfs showed both retinal and choroidal pigmentation.

Thus it seems as if this character were merely a form of expression of the factor for piebald spotting, and that the choroid is just one of those regions, like the face and the belt, in which this factor eliminates color. It certainly shows no association with any other factor.

DISCUSSION AND CONCLUSION.

In a systematic attempt to find any possible linkage of waltzing with the other known genetic characters of mice, the pure-bred Japanese waltzing mouse was used as a source of the waltzing factor. Careful study of this race revealed, what had been suspected before, that its progenitor is most likely Wagner's mouse, *M. wagneri* of Central Asia, and not the common house mouse, *M. musculus*. The latter is probably the ancestor of the other varieties of the domesticated mice.

The test for linkage, therefore, involved a species cross and included 5 genetic factors, each of which proved to be independent of every other. Thus 5 chromosomes were involved and their inheritance followed in an interspecific cross. The method of inheritance of these characters shows a definite tendency of the parental set of chromosomes to associate together in the hybrid and to remain grouped in gametogenesis so that the number of offspring showing the original parental characters is in excess of what might be expected with random recombination of factors according to the laws of chance.

This association of chromosomes appears in the species cross only, and disappears in later generations, forming, in the extracted types, normal Mendelian systems in which there is free segregation of all factors. This is in accord with the observations of Moenkhaus (1904), who finds cytological evidence in fishes, for parental chromosomal association in the Fundulus-Menidia cross. This investigator finds that each parental group of chromosomes remains "spatially distinct" in the hybrid and that there is maintained, in the early cleavages at least, a "bilateral symmetry" between the two sets.

Waltzing is independent of the factors for agouti, albinism, pink-eye, dilution, brown, piebald spotting, short-ear and kinky-tail, and is, therefore, produced by a gene located in some other chromosome than those in which any of the above are found. Its inheritance does not follow the rule of sex-linked factors, and therefore its gene is not located in the sex-chromosome.

It is not certain whether or not the character of kinky-tail is the same as that reported by Plate and others. The factor producing it appears to be definitely linked with short-ear. Its inheritance has not been fully analyzed. Its behavior is less erratic and seems to conform to Mendelian expectation better than the character previously reported.

It is hoped that this experiment can be continued with a view to a more complete determination of the relationship of waltzing to those characters which have been insufficiently studied as yet. The writer also hopes to be able to test the association of characters further by including at least 3 and possibly 4 more factors in the original cross.

SUMMARY.

The facts collected and analyzed in this experiment show the following:

1. That the pure race of Japanese waltzing mice has been derived from the wild form, *Mus wagneri*, and that the varietal name of *Mus wagneri rotans* Fortuyn is therefore valid.

2. That in interspecific crosses of animals (mice) as in plants, there is a tendency for each set of parental chromosomes to group together, forming association systems which may materially modify Mendelian ratios.

3. That interspecific hybrids in mice exhibit heterosis to a marked degree, as to size, vitality, longevity, and metabolic and reproductive activities.

4. That the factor for waltzing is neither sex-linked nor linked with the factors for agouti, albinism, pink-eye, dilution, brown, Dutch spotting, short-ear or kinky-tail. Waltzing shows no evidence of association with any lethal factor.

5. That the character, kinky-tail, is definitely linked, either genetically or physiologically, with short-ear.

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TABLE 1.—*Summary of previous observations on linkage in mice.*

	No. Ind.	Exp. Com.	Exp. Recom.	Obs. Com.	Obs. Recom.	Dev.	P.E.	Dev. ÷ P.E.	Author.
Agouti vs:									
Brown	1180	737.5	442.5	777	403	39.5	11.21	3.52	Little and Phillips 1913.
Do.	275	172.0	103.0	172	103	0.0	5.41	0.00	Little 1916.
Do.	55	34.6	20.4	31	24	3.6	2.44	1.47	Little 1913.
Do.	3734	1867	1867	1856	1878	11.0	20.61	0.53	Detlefsen and Roberts 1918.
Do.	245	122.5	122.5	126	119	3.5	5.28	0.66	Detlefsen and Roberts 1918.
Yellow	Complete linkage or allelomorph.								Cuénot, Morgan, Little.
Dilute	1180	737.5	442.5	752	428	14.5	11.21	1.29	Little and Phillips 1913.
Do.	275	172.0	103.0	183	92	11.0	5.41	2.03	Little 1916.
Do.	55	34.6	20.4	33	22	1.6	2.44	0.65	Little 1913.
Piebald	432	215.0	216.0	220	212	4.0	7.01	.57	Wachter 1921.
Do.	783	391.5	391.5	421	362	29.5	9.44	3.12	Dunn 1920.
B-E-White	242	83	83	82	84	1.0	5.25	0.19	Dunn 1920.
Do.	112	56	56	52	60	4.0	3.57	1.12	Dunn 1920.
Do.	114	57	57	70	44	13.0	3.60	3.61	Dunn 1920.
Do.	230	115	115	121	109	6.0	5.11	1.17	Wachter 1921.
Do.	363	181	181	176	186	5.0	6.42	.77	Wachter 1921.
Pink-eye	1180	737.5	442.5	771	409	33.5	11.21	5.98	Little and Phillips 1913.
Do.	96	Reported independent.							Little 1913.
Do.	935	467.5	467.5	474	461	6.5	10.31	0.63	Dunn 1920.
Do.	3734	1867	1867	1804	1930	63.0	20.61	3.05	Detlefsen and Roberts 1918.
Do.	224	112	112	121	103	9.0	5.05	1.75	Detlefsen and Roberts 1918.
Albinism	46	12	12	14	10	2.0	2.29	0.87	Dunn 1920.
Do.	142	Reported complete linkage.							Hagedoorn 1912.
Waltzing									
Brown vs:									
Yellow	164	Reported linkage, see note.							Dunn 1919.
Dilute	1180	737.5	442.5	765	415	27.5	11.21	2.45	Little and Phillips 1913.
Do.	275	172.0	103.0	178	97	6.0	5.41	1.10	Little 1916.
Do.	55	34.6	20.4	35	20	0.4	2.44	0.16	Little 1913.
Do.	96	60.0	36.0	69	27	9.0	3.19	2.82	Little 1913.
Do.	15	9.3	5.7	9	6	1.0	1.30	.76	Castle and Little 1909.
Do.	270	Reported independent.							Durham 1911.

Table 1.—*Summary of previous observations on linkage in mice.—Continued.*

	No. Ind.	Exp. Com.	Exp. Recom.	Obs. Com.	Obs. Recom.	Dev.	P.E.	Dev. ÷ P.E.	Author.
Brown vs:									
Pink-eye	1180	737.5	442.5	802	378	64.5	11.21	5.75	Little and Phillips 1913.
Do.	354	132.6	221.1	136	218	3.4	6.14	0.55	Little 1913.
Do.	96	36.0	60.0	35	61	1.0	3.19	0.31	Little 1913.
Do.	3734	1867	1867	1878	1856	11.0	20.61	0.53	Detlefsen and Roberts 1918.
Do.	318	159	159	161	157	2.0	6.01	0.16	Detlefsen and Roberts 1918.
Do.	15	5.7	9.3	8	7	1.3	1.26	1.03	Castle and Little 1909.
Do.	270	Reported independent.							Durham 1911.
Dilute vs:									
Pink-eye	1180	737.5	442.5	783	387	45.5	11.21	4.05	Little and Phillips 1913.
Do.	137	51.4	85.6	60	77	8.6	3.82	2.25	Little 1913.
Do.	124	46.4	27.6	50	24	3.6	3.63	0.99	Little 1913.
Do.	96	36.0	60.0	38	58	2.0	3.19	0.62	Little 1913.
Do.	15	5.7	9.3	8	7	2.3	1.30	1.76	Castle and Little 1909.
Piebald vs.									
Black-eyed-white	449	224.5	224.5	231	218	6.5	7.15	0.90	Dunn 1920.
Do.	44	22	22	22	22	0.0	2.24	0.0	Little 1917.
Do.	202	101	101	108	96	7.0	4.79	1.46	Little 1917.
Do.	57	28.5	28.5	31	26	2.5	2.55	0.98	Little 1915.
Do.	128	64	64	54	74	10.0	3.82	2.61	Little 1915.
Do.	423	211.5	211.5	218	205	6.5	6.98	0.93	So and Imai 1919.
Pink-eye	774	290.2	483.8	295	479	4.8	9.08	0.51	Dunn 1920.
Do.	1172	586	586	623	547	37.0	11.54	3.20	Dunn 1920.
Albinism	474	88.8	....	90	....	1.2	5.72	0.20	Durham 1908.
Do.	44	24.75	8.25	21	9	2.75	1.94	1.90	Cuénot 1907.

TABLE 1.—*Summary of previous observations on linkage in mice.—Continued.*

	No. Ind.	Exp. Com.	Exp. Recom.	Obs. Com.	Obs. Recom.	Dev.	P.E.	Dev.÷ P.E.	Author.
Black-eyed White vs:									
Pink-eye	82	41	41	39	43	2.0	3.05	0.65	Dunn 1920.
Do.	62	15.5	15.5	18	13	2.5	2.66	0.93	Dunn 1920.
Do.	59	16.0	16.0	11	21	5.0	2.59	1.93	Dunn 1920.
Do.	80	44.0	40.0	38	42	2.0	3.02	0.66	Dunn 1920.
Do.	77	38.5	38.5	46	31	7.5	2.96	2.53	Wachter 1921.
Do.	281	140.5	140.5	134	147	6.5	5.65	1.15	Wachter 1921.
Pink eye vs:									
Albinism	58	108.75	326.25	120	295	31.25	7.03	4.44	Dunn 1920.
Do.	3141	....	785.25	....	222	563.25	16.4	34.34	Dunn 1920.
Do.	3331	832.75	....	1414	....	581.25	16.8	34.59	Dunn 1920.
Do.	555	104.4	313.6	131	287	26.6	6.88	3.86	Darbishire 1904.
Do.	3142	....	785.5	....	222	563.5	18.90	29.81	Castle 1919.
Do.	63	32.5	32.5	45	18	12.5	2.68	4.66	Castle 1919.
Do.	75	37.5	37.5	55	20	17.5	2.92	5.99	Castle 1919.
Do.	227	42.6	127.7	est 51.7	111	16.7	4.40	3.79	Haacke 1915.
Do.	3880	....	....	Cross-over per cent 19.06 and 13.89.					Castle and Wachter 1924.
Do.	765	143.4	430.3	159	390	15.6	8.08	1.93	Detlefsen 1925.
Do.	2544	....	636.0	....	197	439.0	17.00	25.8	Detlefsen 1925.
Do.	1964	491.0	....	909	....	416.0	14.90	27.9	Detlefsen 1925.
Paleness	1137	284.25	....	523	....	238.75	11.46	20.83	Detlefsen and Clements 1924.
Do.	474	237.0	237.0	409	65	172.0	7.34	23.44	Detlefsen and Clements 1924.
Waltzing	555	260.1	156.0	274	144	14.0	7.69	1.82	Darbishire 1904.
Albinism vs:									
Waltzing	555	208.2	346.8	191	364	17.2	7.69	2.22	Darbishire 1904.
Do.	779	292.2	486.8	280	499	12.2	9.11	1.33	Wachter 1921.

TABLE 2.—*Comparative measurements in mm. of Mus wagneri, the Japanese waltzing mouse, the house mouse and certain hybrid strains.*

	Wagneri (Hilzheimer).	Wagneri (Harvard Museum).	Japanese.	Wild.	Fancy.	Pure pdb.	F_1 Jap x pdb.	Extracted PDBsw.	Extracted pdbSW.	Miscellaneous Waltzers.
1 No. ind.	2	2–13	6	6	6	6	6	6	6	12
2 Head and body	80.0	80.0	85.3	100.9	103.0	96.8	98.2	85.8	93.2	91.0
3 Tail	55.0	57.3	60.0	86.7	87.1	86.2	76.2	73.7	80.8	74.7
4 Ratio, tail : total	40.7 *p. ct.*	41.8 *p. ct.*	41.3 *p. ct.*	46.2 *p. ct.*	45.8 *p. ct.*	47.1 *p. ct.*	43.5 *p. ct.*	46.1 *p. ct.*	46.4 *p. ct.*	44.0 *p. ct.*
5 Head	21.5	......	24.2	24.5	26.0	25.3	26.5	24.2	24.8	24.8
6 Front of eye to tip of muzzle	9.5	......	10.2	10.9	11.1	11.2	11.8	10.2	10.8	10.5
7 Front of eye to front of ear	10.8	......	9.7	11.9	11.5	10.7	12.4	10.7	11.0	10.8
8 Fore foot (elbow to tip of toes)	9.0?	15.2	15.3	20.4	20.2	20.2	21.1	19.7	20.4	20.8
9 Hind foot (heel to tip of toes)	15.5?	14.0	14.0	17.6	17.2	17.8	16.2	16.7	16.9	17.7
10 Ear length	9.0	......	9.2	10.6	10.6	11.6	12.3	10.3	10.3	10.0
11 Ear width	9.0	......	10.3	11.0	11.7	11.7	12.6	10.6	10.4	10.6
12 Longest vibrissa	20.0	......	21.6	27.3	24.5	25.1	24.2	22.3	24.7	22.6
13 Basilar	......	15.2	16.0	17.9	17.7	18.0	19.0	16.4	16.8	16.6
14 Condylo-basal	......	18.5	20.3	21.9	22.0	22.1	23.8	21.3	21.8	21.8
15 Palatilar	......	7.8	8.5	9.8	10.0	10.2	10.8	9.4	9.6	9.4
16 Basilo-palatilar	......	7.3	7.4	8.1	7.8	8.0	8.3	7.5	7.4	7.2
17 Interdental gap	......	4.4	5.2	5.8	5.7	5.9	6.5	5.5	5.3	5.3
18 3 Cheek teeth alveoli	......	3.6	3.3	3.6	3.5	3.6	3.7	3.3	3.5	3.2
19 Outside orbits	......	10.8	10.4	11.4	11.9	12.0	12.0	11.0	11.4	11.0
20 Outside parietals	......	9.8	9.3	10.2	10.3	10.2	9.9	9.7	9.9	9.8
21 Ratio, width: length	......	48.3 *p. ct.*	51.2 *p. ct.*	52.0 *p. ct.*	54.1 *p. ct.*	54.3 *p. ct.*	50.4	51.8	51.1	52.7
22 Depth, foramen mag.	......	3.3	3.3	3.6	3.9	3.8	3.6	3.7	3.5	3.6
23 Width, foramen mag.	......	3.7	3.5	4.1	4.4	4.5	3.8	4.0	4.2	3.9
24 Depth of skull	......	6.5	6.2	6.7	7.1	6.8	6.9	6.3	6.6	6.3
25 No. tail vert.	19–20	19–20	21–22	26–28	27–28	23–25	23–25	24–26	25–28	23–28
26 10th tail vert.	......	2.4	3.3	4.4	4.8	4.6	4.0	3.8	4.2	4.0
27 No. scale rings	136.9	......	141.3	175.1	180.8	175.0	170.1	151.2	165.7	163.3
28 Ditto per mm.	2.4	......	2.3	2.0	2.0	2.0	2.2	2.0	2.0	2.1

TABLE 3.—*Precipitin reactions. Numbers indicate time in minutes.*

Dilution.	Wagneri dried.		Japanese				Fancy,				Rat, dried.	
			Dried.		Fresh.		Dried.		Fresh.			
	Owl.	Hen.	Owl.	Hen.	Owl.	Hen.	Owl.	Hen.	Owl.	Hen.	Owl.	Hen.
1 : 2............	1½	1½	3	3	3	3	⅓	¼	¼	¼	5	8
1 : 4............	2	3	3½	4	4	4	⅓	½	½	½	15	15
1 : 8............	10	10	8	12	12	10	2	2	3	2	25	25
1 : 16...........	25	30	30	30	35	30	5	10	10	8	40	45

TABLE 4.—*Expected and observed frequencies of recombination groups of characters among F_2 and back-cross descendants of Japanese waltzing mice mated with house mice.*

	Expected.		Obtained.		Deviation.		Probable error.		Deviation ÷ probable error.	
	F_2	Back-cross.	F_2	Back-cross.	F_2	Back-cross.	F_2	Back-cross.	F_2	Back-cross.
PDBs.	120	117.6	151	139	31	21.4	7.28	6.71	4.14	3.1
PDBw.	120	117.6	161	150	41	32.4	7.28	6.71	5.63	4.8
PDsw.	40	117.6	71	143	31	25.4	4.84	6.71	6.40	3.7
PBsw..	40	117.6	68	123	28	5.4	4.84	6.71	5.78	.8
Dbsw..	40	80.5	63	117	23	36.5	4.84	5.80	4.7	6.2
PDB..	481	235.2	522	264	41	28.8	11.06	8.97	3.7	3.2
PDs...	160	235.2	191	251	31	15.8	7.89	8.97	3.9	1.7
PDw..	160	235.2	225	269	65	33.8	7.89	8.97	8.2	3.7
PBs...	160	235.2	171	225	11	10.2	7.89	8.97	1.3	1.1
PBw..	160	235.2	191	247	31	11.8	7.89	8.97	3.9	1.3
DBs.,.	160	161.0	159	194	1	33.0	7.89	8.02	.1	4.1
DBw..	160	161.0	168	202	8	41.0	7.89	8.02	.9	5.1
Psw...	53	235.2	85	252	32	16.8	4.78	8.97	3.5	1.9
Dsw...	53	161.0	78	194	25	33.0	4.78	8.02	3.1	4.1
Bsw...	53	161.0	71	173	18	12.0	4.78	8.02	2.2	1.4

TABLE 5.—*Expected and observed numbers of each class in each cross.*

A, a = agouti, non-agouti. D, d = density, dilution. S, s = self, spotting.
B, b = black, brown. K, k = straight, kinky tail. Se, se = normal ear, short ear.
C, c = color, albinism. P, p = pigmented, pink-eye. W, w = normal gait, waltzing

Classes.	Expected.	Observed.	Classes.	Expected.	Observed.
PDBSW.....	58.8	61	CW.........	38	20
PDBSw......	58.8	64	Cw..........	38	37
PDBsW......	58.8	53	cW..........	38	46
PDbSW......	58.8	36	cw..........	38	49
PdBSW......	58.8	61	reciprocal.....	...	...
PDBsw......	58.8	86	CW.........	88	83
PDbsW......	58.8	55	Cw..........	88	77
PdbSW......	58.8	62	cW..........	88	86
PdBSw......	58.8	60	cw..........	88	106
PDbSw......	58.8	62	....	...	...
PdBsW......	58.8	49	ASW........	13.8	18
PDbsw.......	58.8	57	ASw.........	13.8	17
PdbsW.......	58.8	66	AsW.........	13.8	13
PdbSw.......	58.8	60	aSW.........	13.8	13
PdBsw.......	58.8	37	Asw	13.8	24
Pdbsw.......	58.8	72	asW.........	13.8	8
pDBSW......	21.7	20	aSw.........	13.8	5
pDBSw......	21.7	21	asw.........	13.8	13
pDBsW......	21.7	24	....	...	...
pDbSW......	21.7	23	SeKW.......	86.4	110
pdBSW......	21.7	29	SeKw........	28.8	26
pDBsw......	21.7	31	SekW........	28.8	13
pDbsW......	21.7	18	seKW.......	28.8	6
pdbSW......	21.7	24	Sekw.........	9.6	2
pdBSw.......	21.7	26	sekW........	9.6	37
pDbSw.......	21.7	17	seKw........	9.6	4
pdBsW......	21.7	18	sekw.........	3.2	8
pDbsw.......	21.7	20	....	...	...
pdbsW.......	21.7	17	SeK.........	57.6	75
pdbSw.......	21.7	25	Sek..........	19.2	5
pdBsw.......	21.7	19	seK.........	19.2	0
pdbsw.......	21.7	16	sek..........	6.4	23

TABLE 6.—*Frequencies of recessive factors in relation to expectation.*

	No. Ind.	Expected.	Observed.	Deviation.	Probable error.	Dev. ÷ P.E.
Pink-eye..............	1289	322.2	348	+25.8	10.45	2.46
Dilution...............	1289	644.5	641	— 3.0	12.07	0.24
Brown................	1289	644.5	630	—14.5	12.07	1.20
Spotting...............	1400	700.0	696	— 4.0	12.61	0.31
Non-agouti............	111	55.5	39	—16.5	3.55	4.64
Short-ear.............	309	77.2	78	+ .8	5.13	0.15
Kinky-tail............	309	77.2	88	+10.8	5.13	2.10
Albinism..............	504	252.0	287	+35.0	7.57	4.62
Waltzing B.C...........	1289	644.5	673	+28.5	12.07	2.36
Do..................	111	55.5	59	+ 3.5	3.55	0.98
Do..................	504	252.0	269	+17.0	7.57	2.24
Waltzers total..........	1904	952.0	1001	+49.0	14.70	3.33
Waltzers F_2............	1247	311.7	353	+41.3	10.25	4.02
Waltzers F_2............	206	51.5	40	—11.5	4.19	2.74
Waltzers total F_2.......	1455	363.2	393	+29.8	11.12	2.68
Waltzers grand total....	3359	1315.2	1394	+78.8	....	....

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TABLE 7.—*Frequencies of character pair recombinations, with reference to linkage.*

	No. Ind.	Expected parental combinations.	Expected recombinations.	Observed parental combinations.	Observed recombinations.	Deviation.	Probable error.	Dev. ÷ P.E.
Pink-eye—dilution	348	174.0	174.0	174	174	0.0	6.29	0.0
Pink-eye—brown	348	174.0	174.0	160	188	+14.0	6.29	2.22
Pink-eye—spotting	348	174.0	174.0	185	163	—11.0	6.29	1.74
Dilution—brown	1289	644.5	644.5	702	587	—57.5	12.07	4.76
Dilution—spotting	1289	644.5	644.5	691	598	—46.5	12.07	3.85
Brown—spotting	1289	644.5	644.5	626	663	+18.5	12.07	1.53
Non-agouti—spotting	111	55.5	55.5	56	55	— .5	3.55	0.14
Short-ear—kinky-tail	309	193.0	115.8	279	30	—86.0	5.73	15.01
Waltzing—pink-eye	348	174.0	174.0	173	175	+ 1.0	6.29	0.15
Waltzing—dilution	1289	644.5	644.5	684	605	—39.5	12.07	3.27
Waltzing—brown	1289	644.5	644.5	645	644	— .5	12.07	0.04
Waltzing—spotting	1400	700.0	700.0	722	678	—22.0	12.61	1.74
Waltzing—non-agouti	111	55.5	55.5	49	62	+ 6.5	3.55	1.83
Waltzing—short-ear	206	77.4	129.0	71	135	+ 6.4	4.65	1.37
Waltzing—kinky-tail	206	77.4	129.0	80	126	+ 2.6	4.65	0.55
Waltzing—albino	504	252.0	252.0	272	232	—20.0	7.57	2.64
Waltzing—albino F_2	101	75.75	25.25	79	22	— 3.25	2.94	1.1

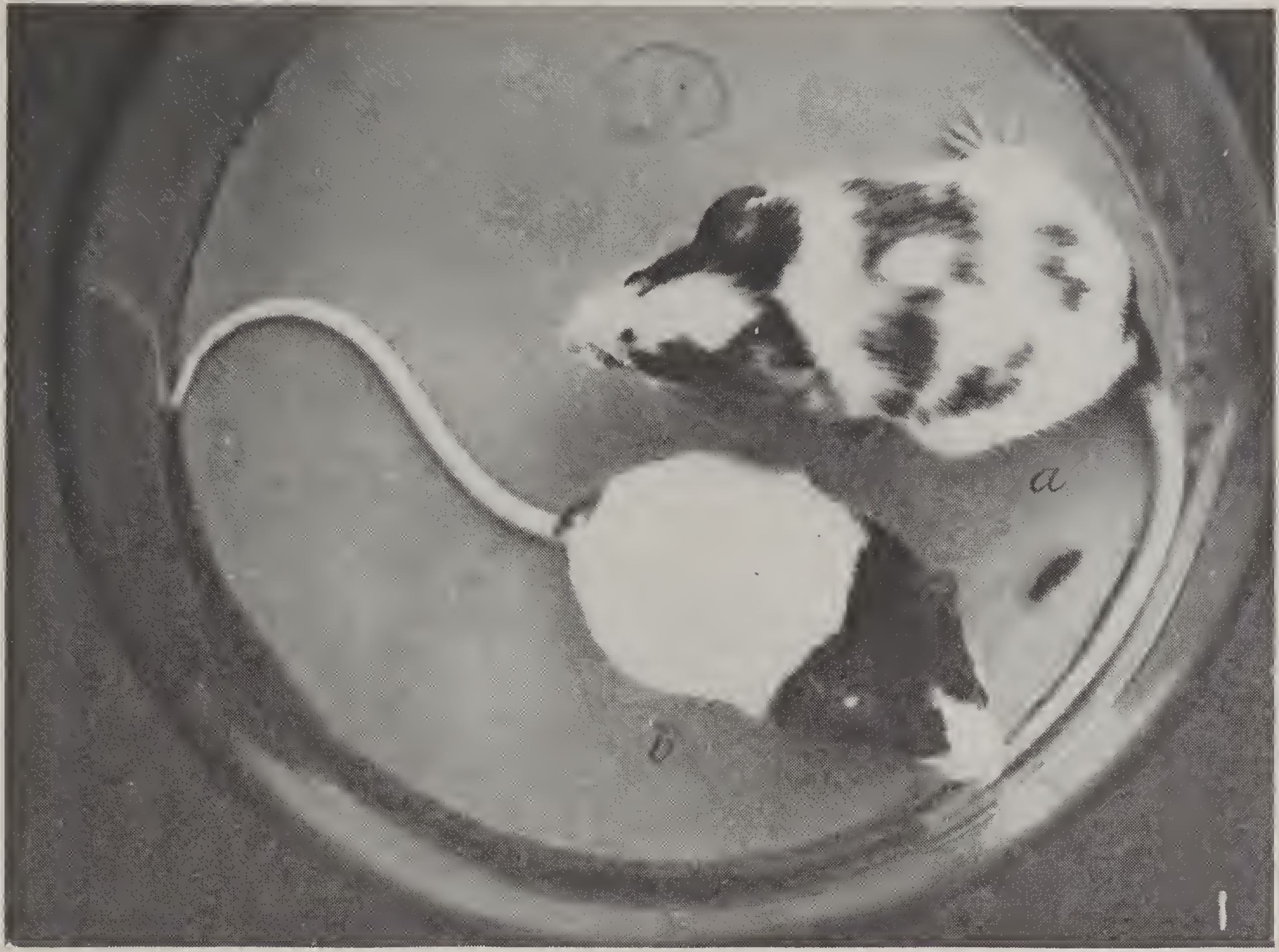

FIG. 1.—Types of spotting (*a*) dominant and (*b*) recessive. Mouse (*a*) transmitted both black-eyed-white and piebald characters; mouse (*b*) only piebald, the kind of spotting found in the Japanese waltzer.

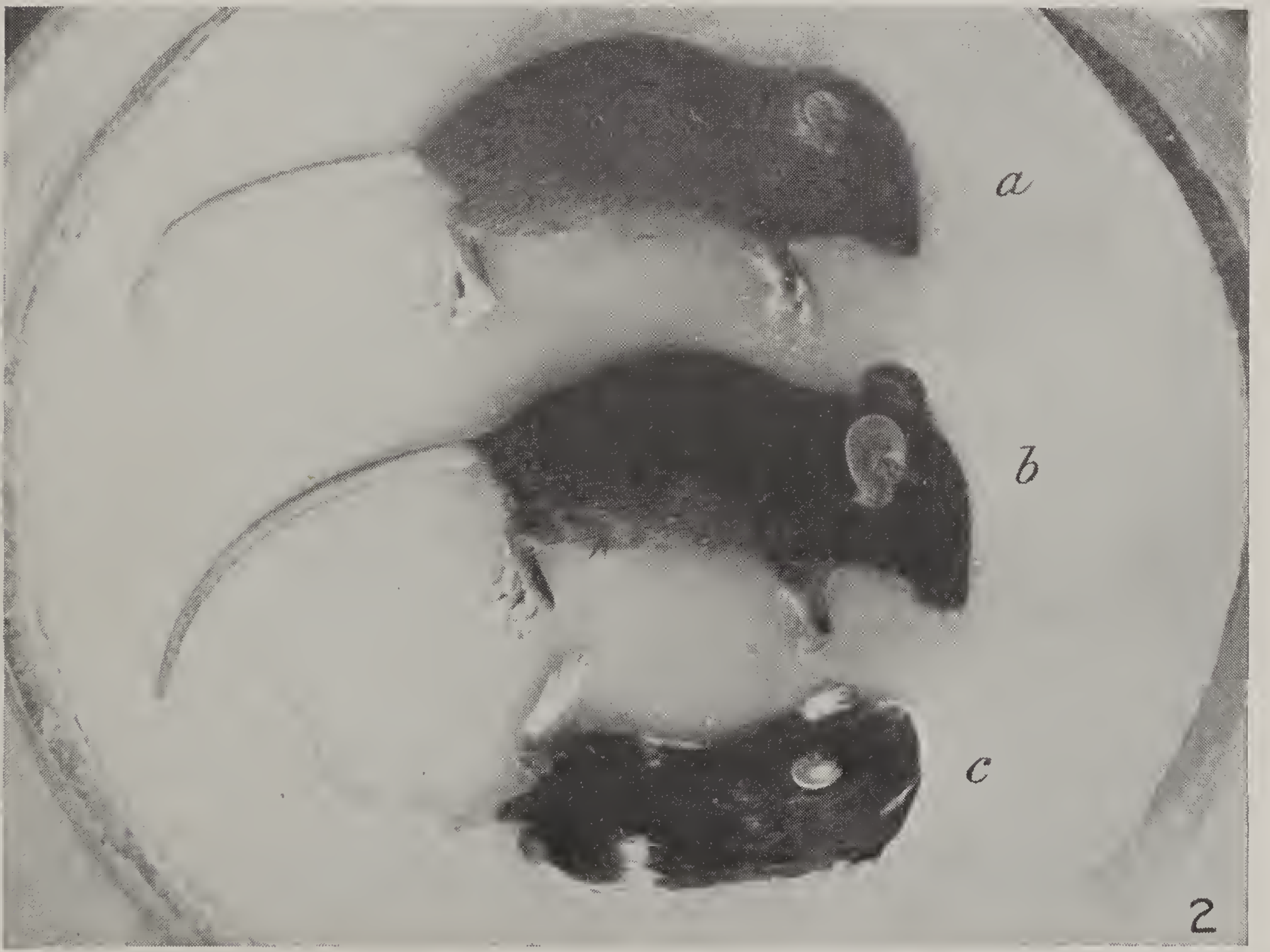

FIG. 2.—Short-ear and kinky-tail (*a* and *c*), normal (*b*). *a* and *b* litter mates, 18 days old; *c* younger. Slightly reduced.

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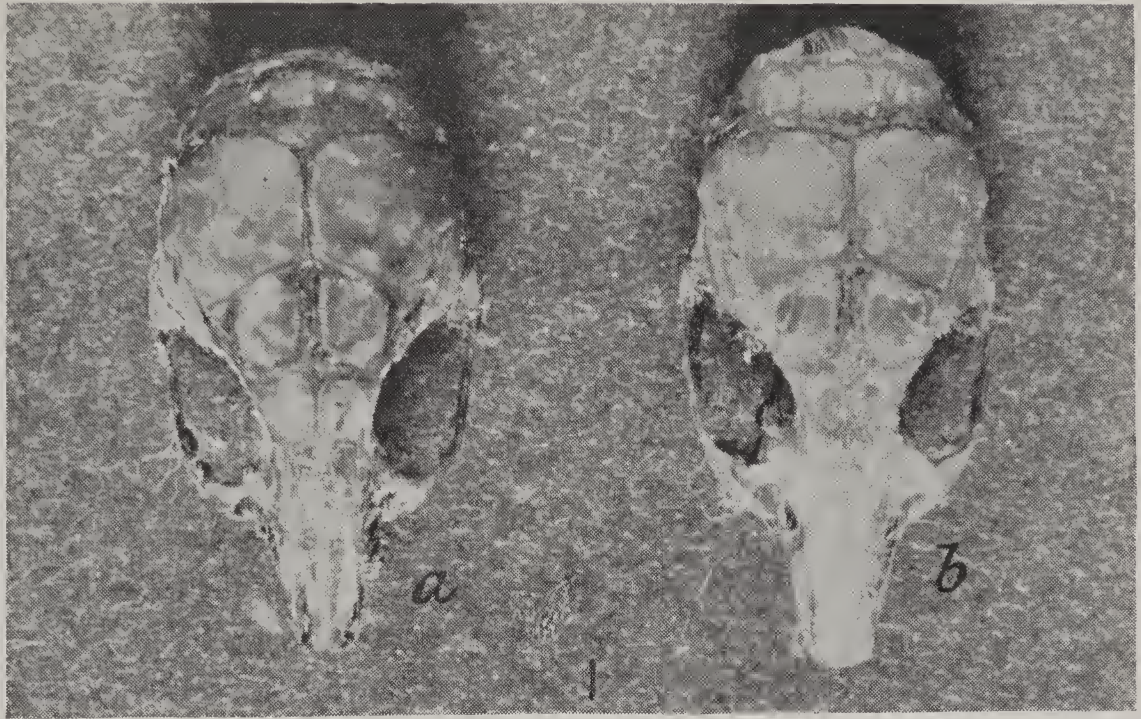

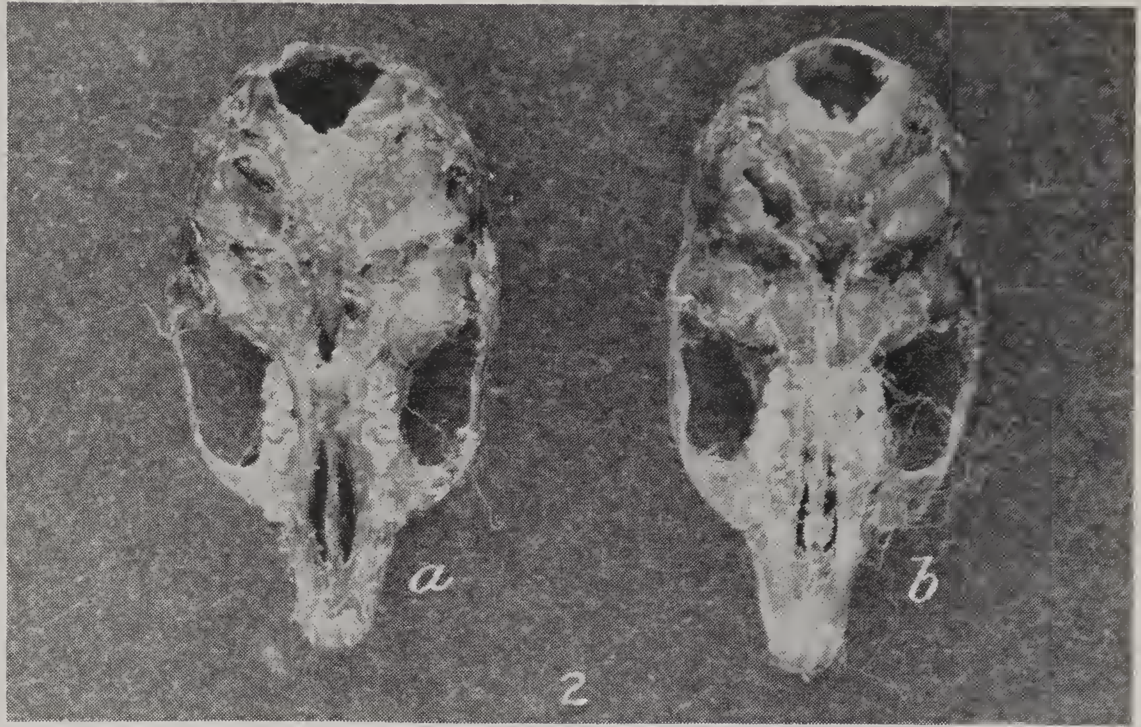

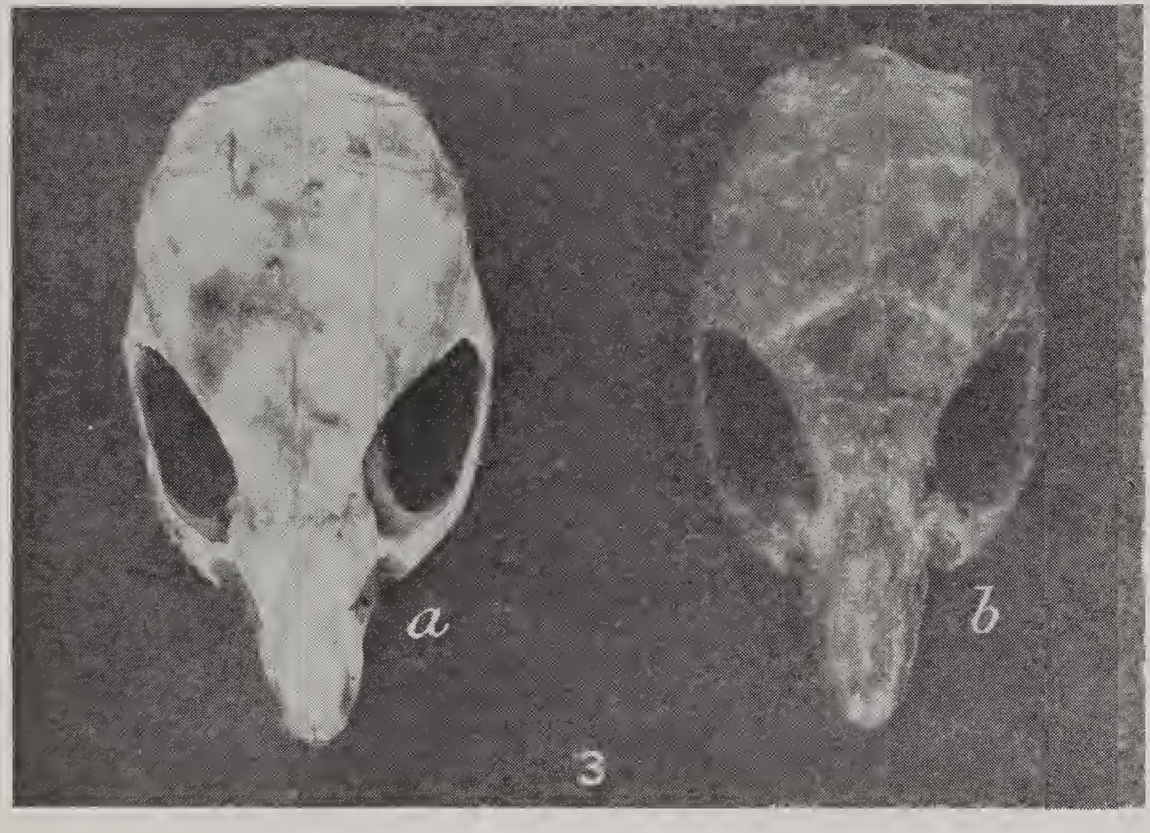

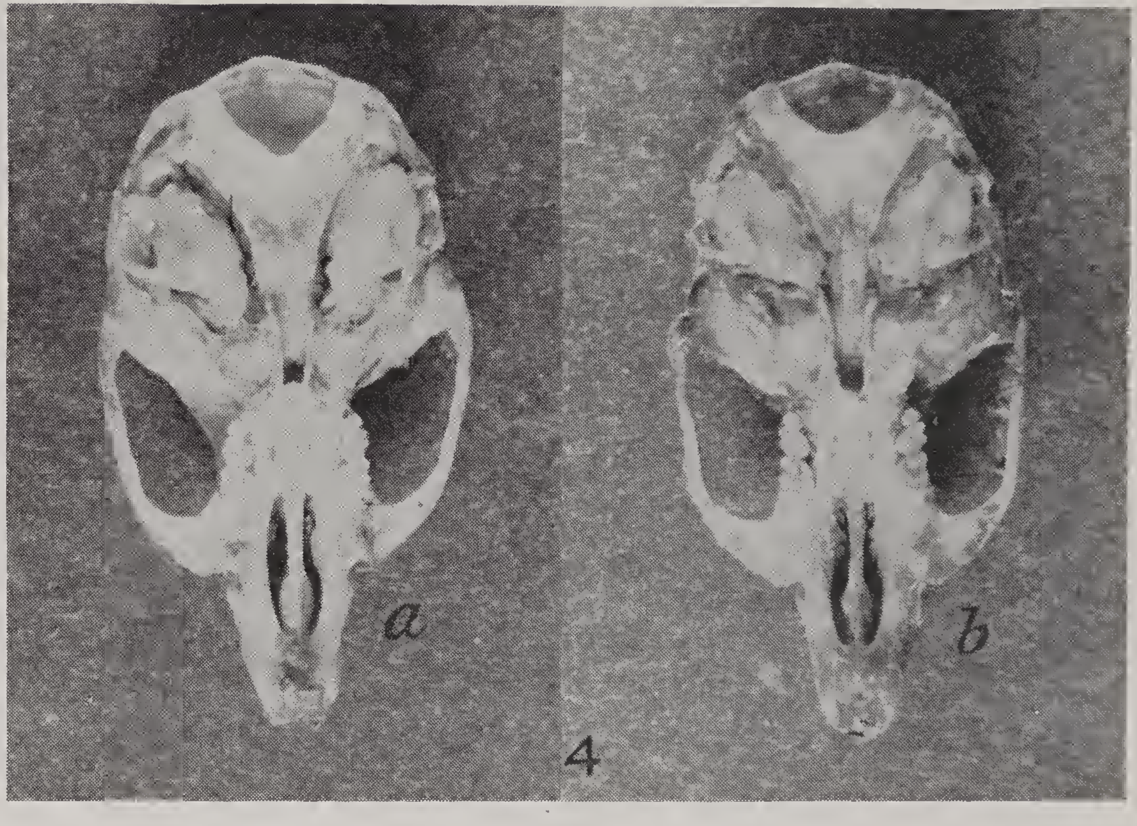

Fig. 1.—Skulls of Wagner's mouse (*a*) and Japanese waltzer (*b*), dorsal view. 2.5 times natural size.
Fig. 2.—Same skulls as figure 1, ventral view. Same enlargement.
Fig. 3.—Skulls of wild house-mouse (*a*) and fancy mouse (*b*), dorsal view. 2.5 times natural size.
Fig. 4.—Same skulls as figure 3, ventral view. Same enlargement.

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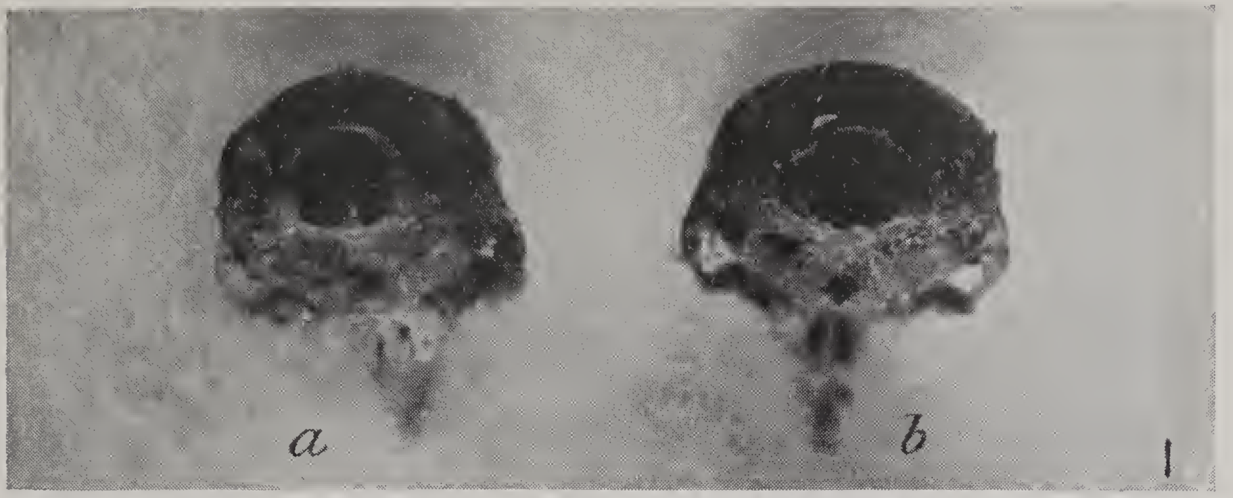


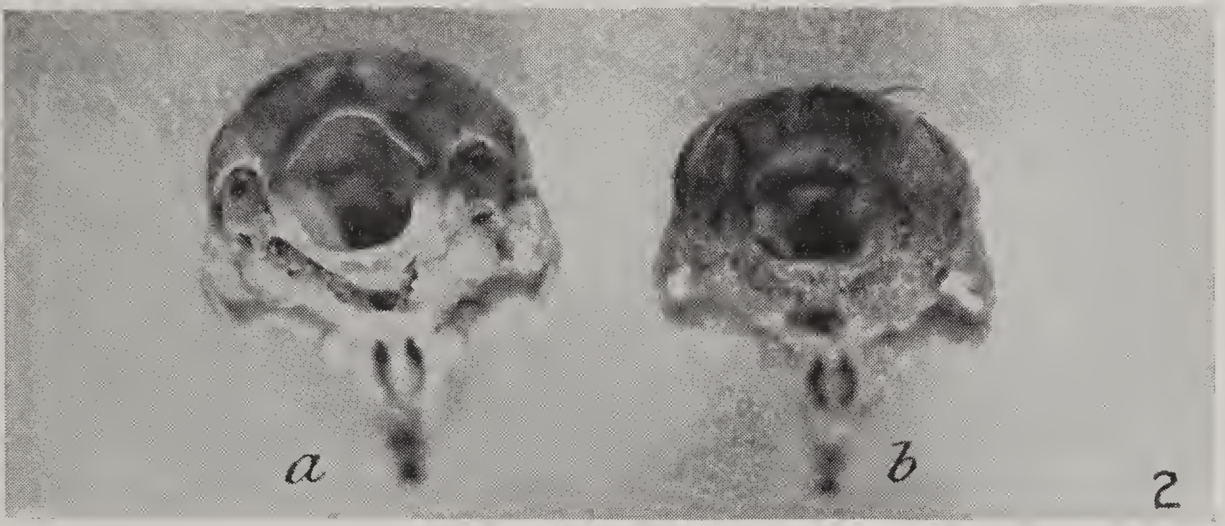
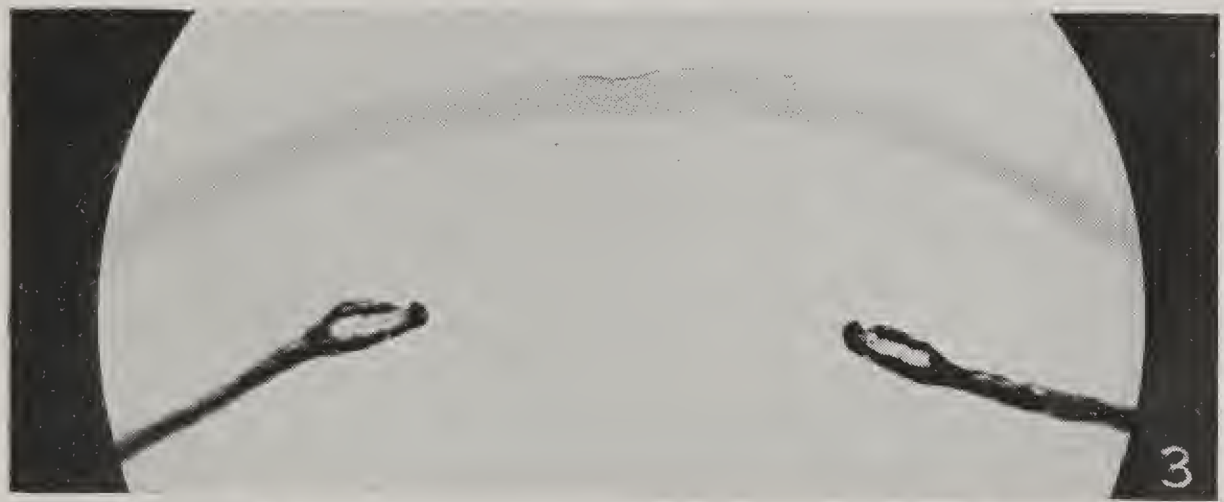


FIG. 1.—Skulls of Wagner's mouse (*a*) and Japanese waltzer (*b*), posterior view. 2.5 times natural size. Compare Plate 2, figures 1 and 2.

FIG. 2.—Skulls of wild house-mouse (*a*) and fancy mouse (*b*), posterior view. 2.5 times natural size. Compare Plate 2, figures 3 and 4.



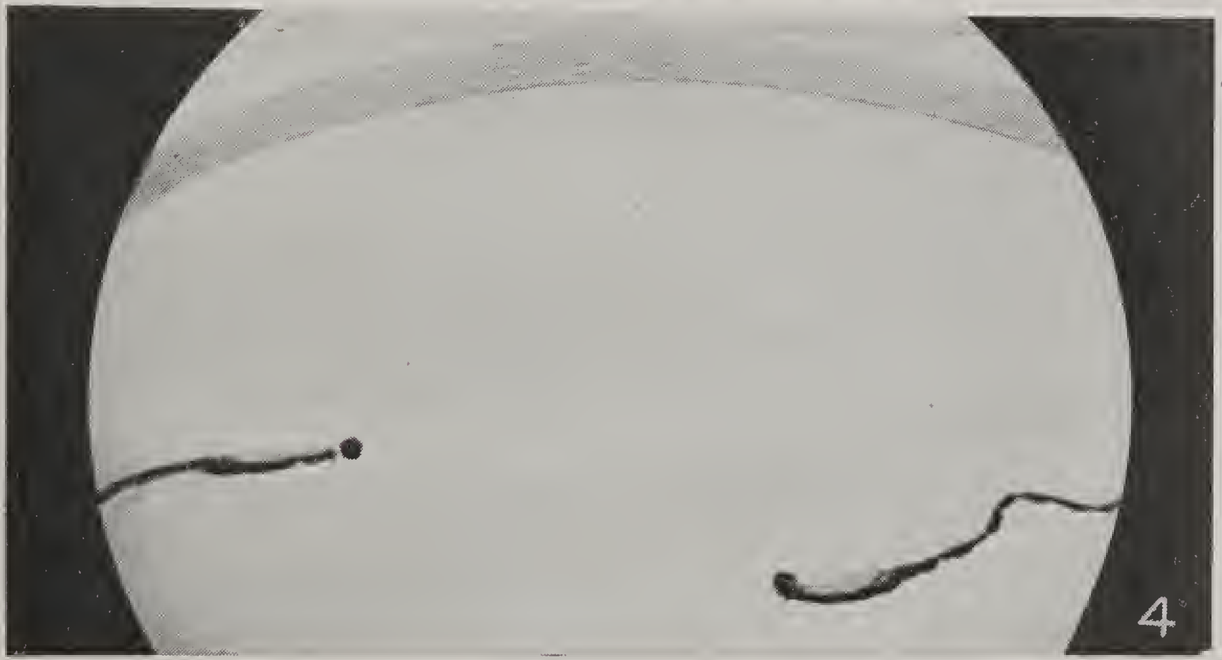


FIG. 3.—Cross-section of iris of F_1 hybrid between (1) Jap and (2) pink-eyed, dilute brown. Note pigment in front of as well as behind sphincter muscle. Photomicrograph.

FIG. 4.—Cross-section of iris of Japanese waltzer, showing pigment behind sphincter muscle only. Photomicrograph.

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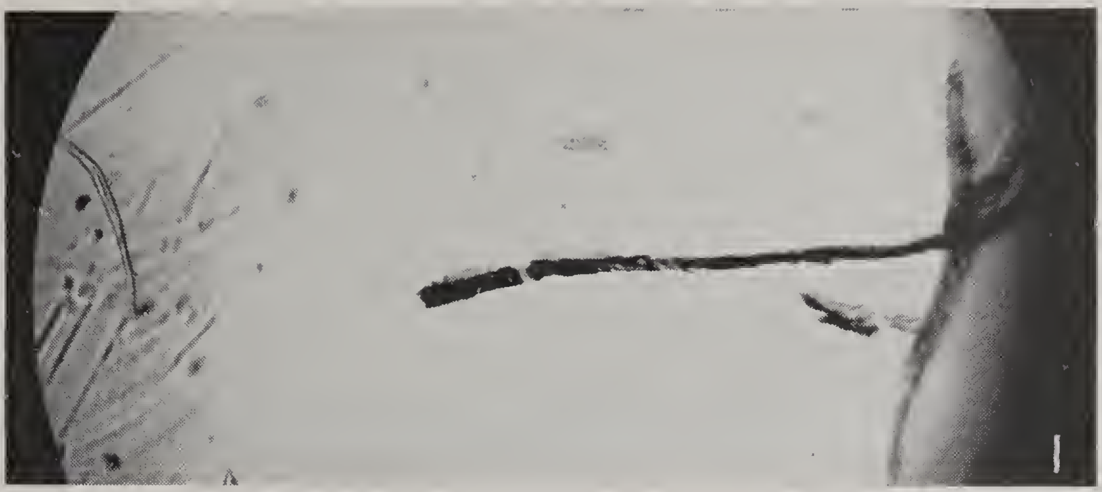
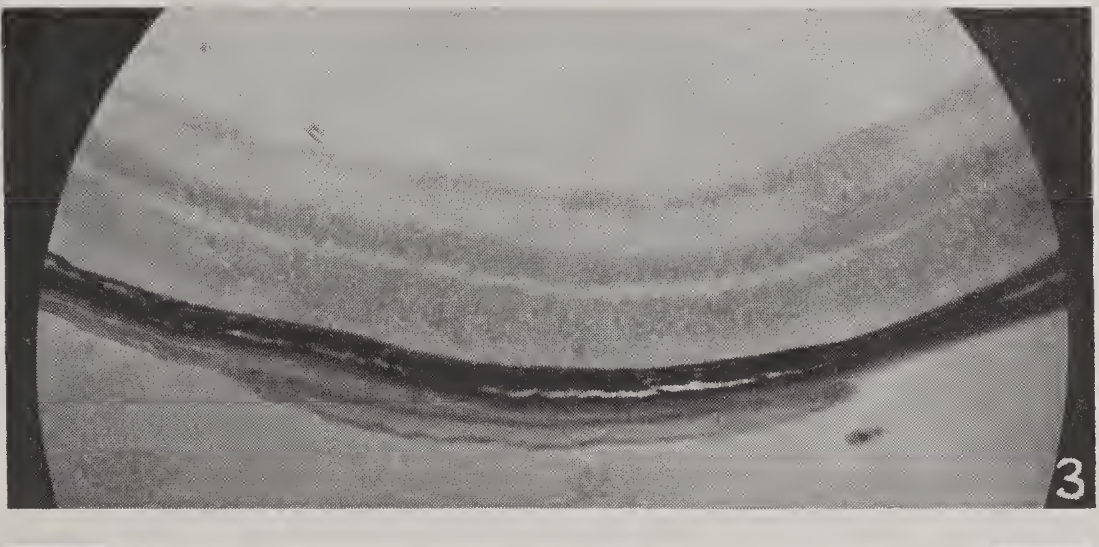


Fig. 1.—Cross-section of iris of Wagner's mouse, showing pigment behind sphincter muscle only, as in Japanese waltzer.

Fig. 2.—Same as in figure 1, other eye of same mouse.



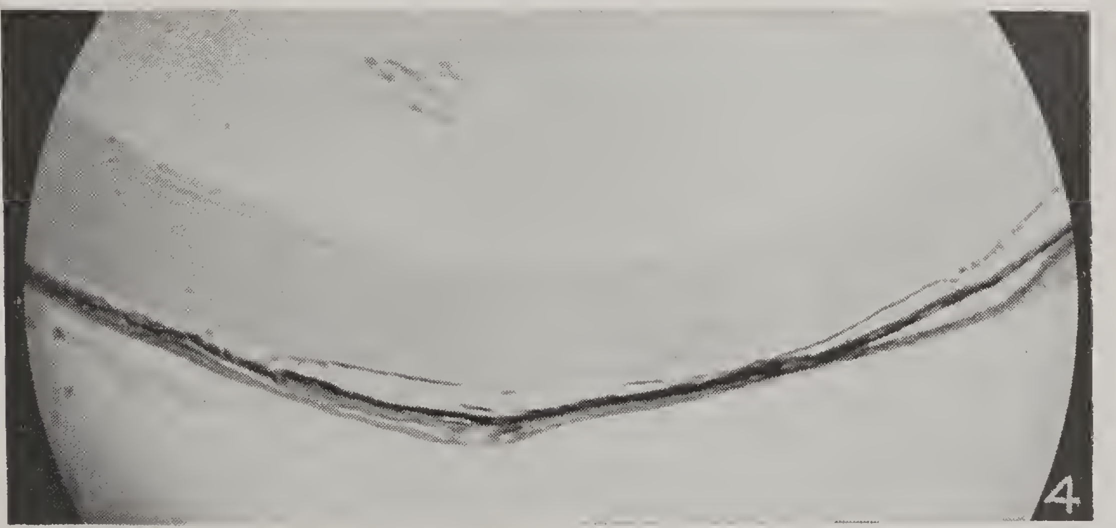


Fig. 3.—Cross-section of wall of eye of F_1 hybrid between (1) Japanese waltzer and (2) pink-eyed, dilute brown. Note both retinal and choroidal pigment separated by basal membrane.

Fig. 4.—Similar cross-section of wall of eye of pure Japanese waltzer, showing retinal pigment only.

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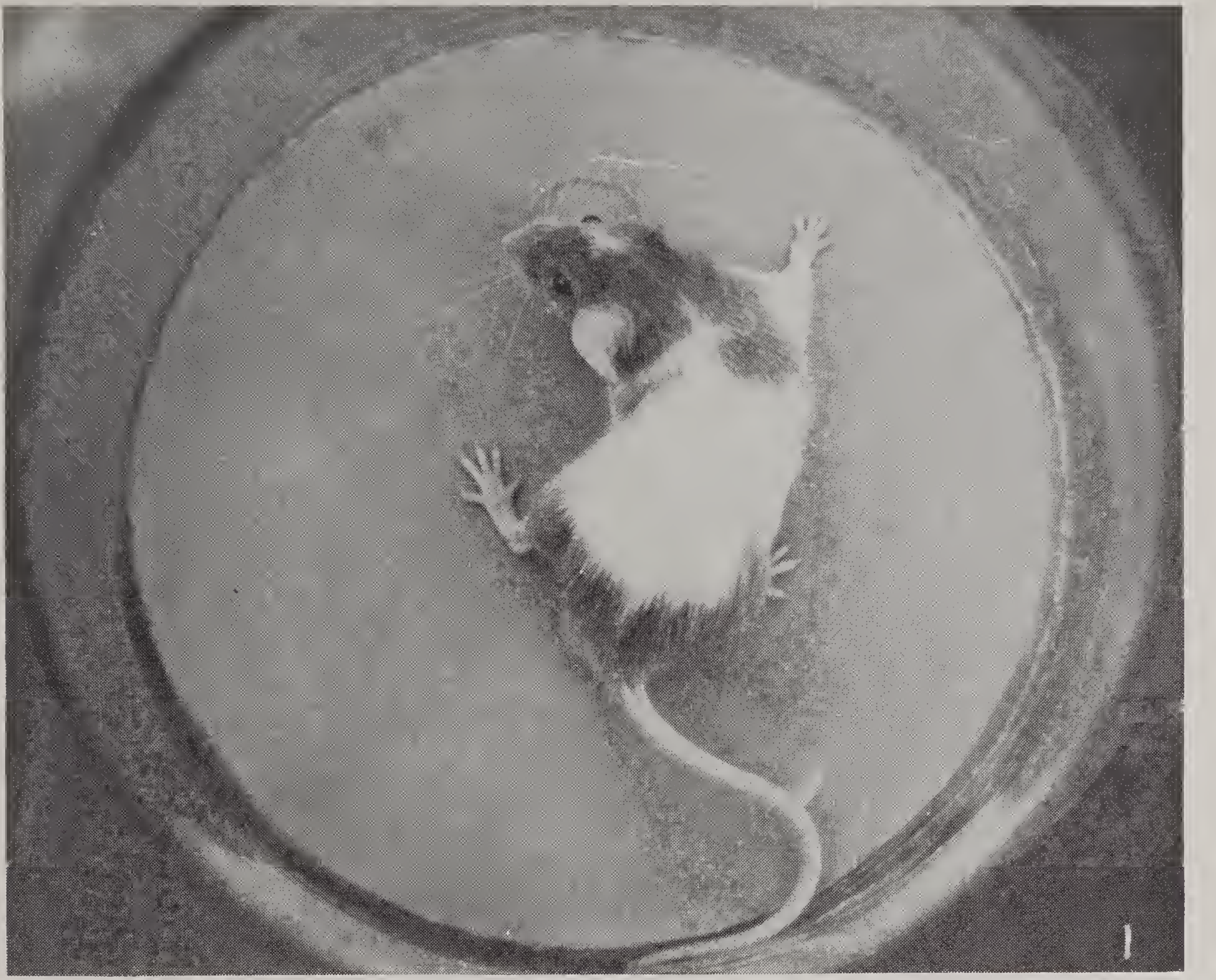

FIG. 1.—Male No. 2011, an extracted dilute, brown, spotted waltzer. Type of male used in back-crosses, showing extracted form of recessive (piebald) spotting. Slightly reduced.

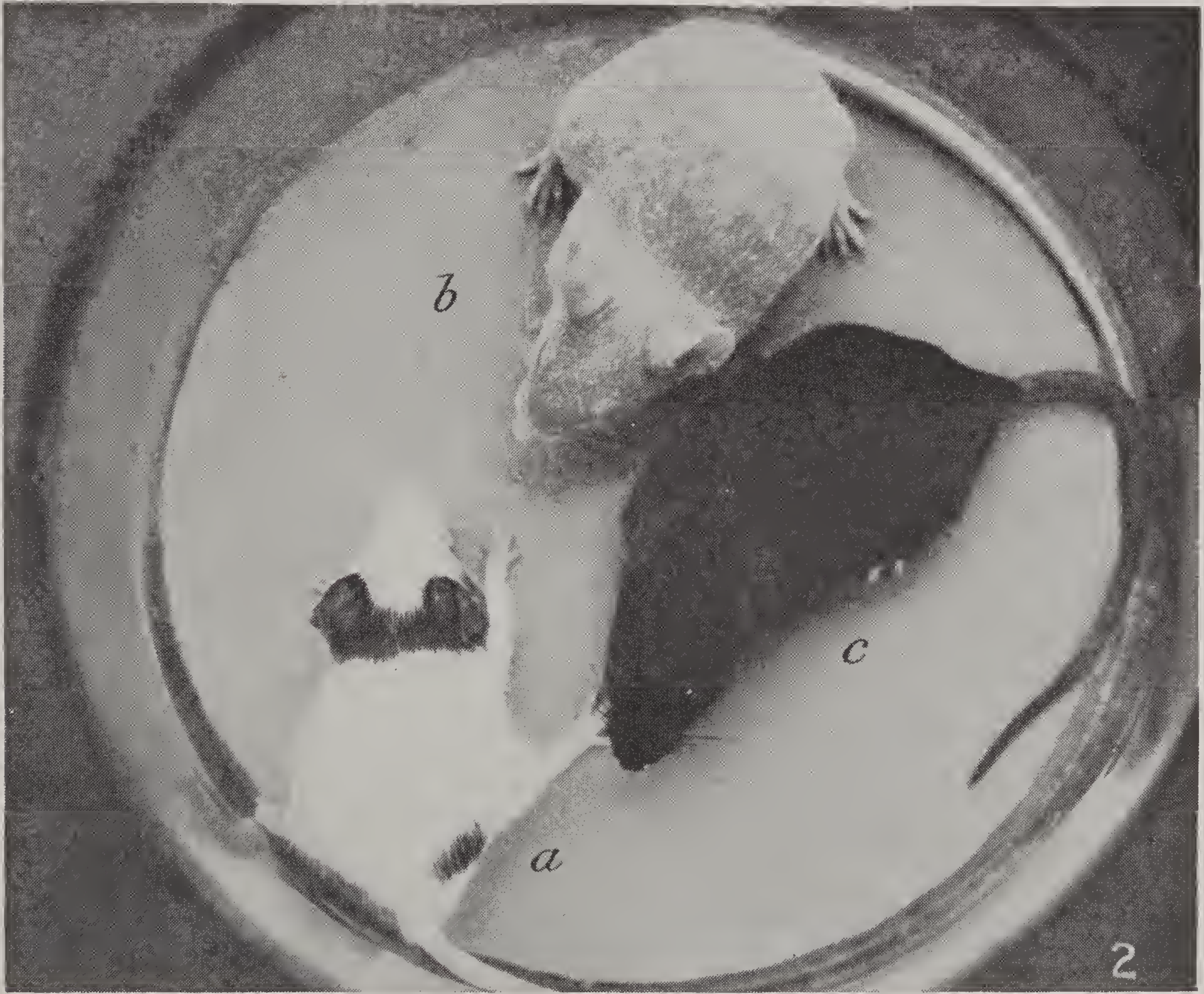

FIG. 2.—(*a*) Pure Japanese waltzer, (*b*) pink-eyed, dilute brown, (*c*) F_1 hybrid between (*a*) and (*b*). Slightly reduced.

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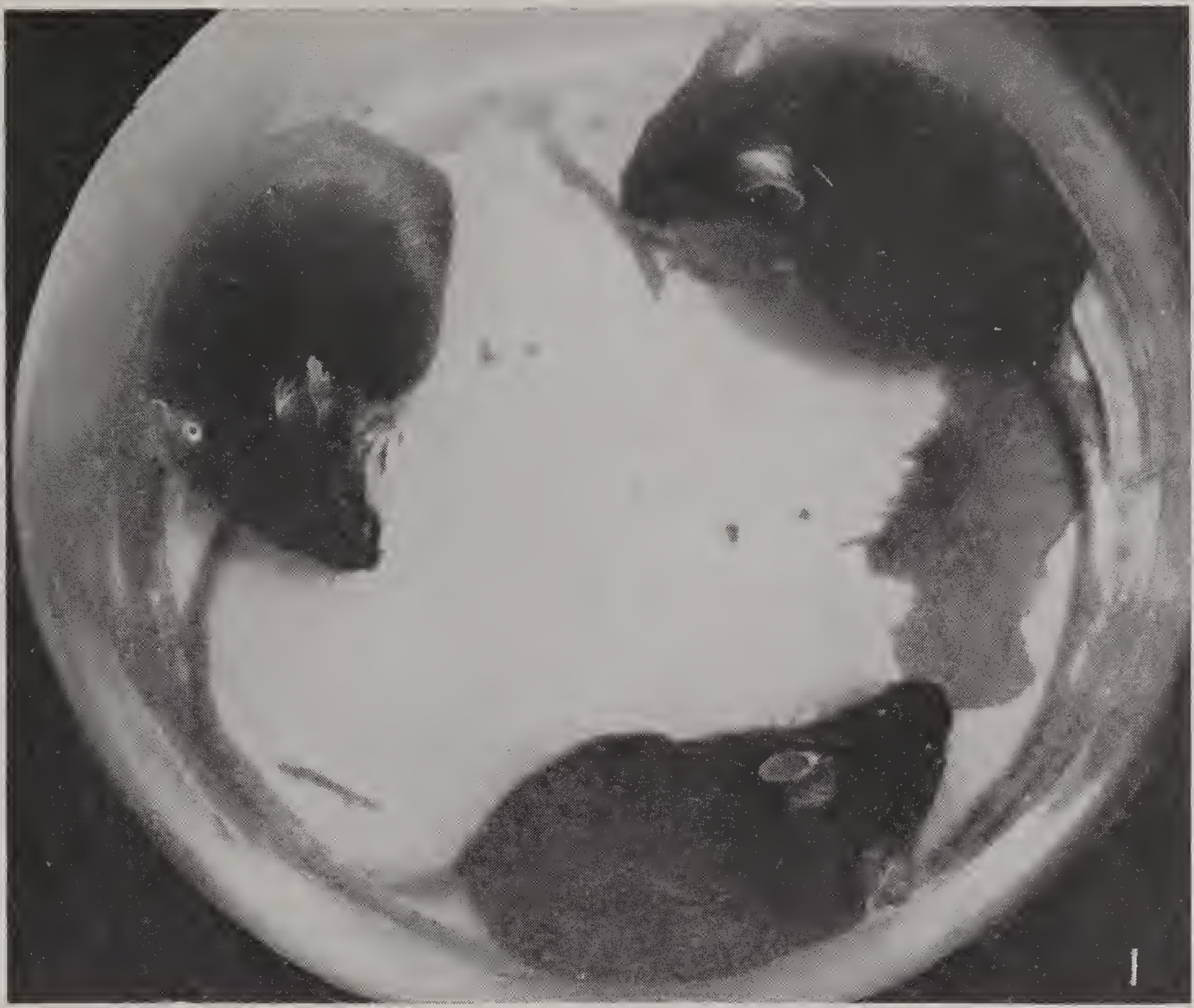

Fig. 1.—F_1 hybrid females born July 1923, photographed July 25, 1925: Note vigorous condition at age of two years. White hairs are appearing throughout pelage. Slightly reduced.

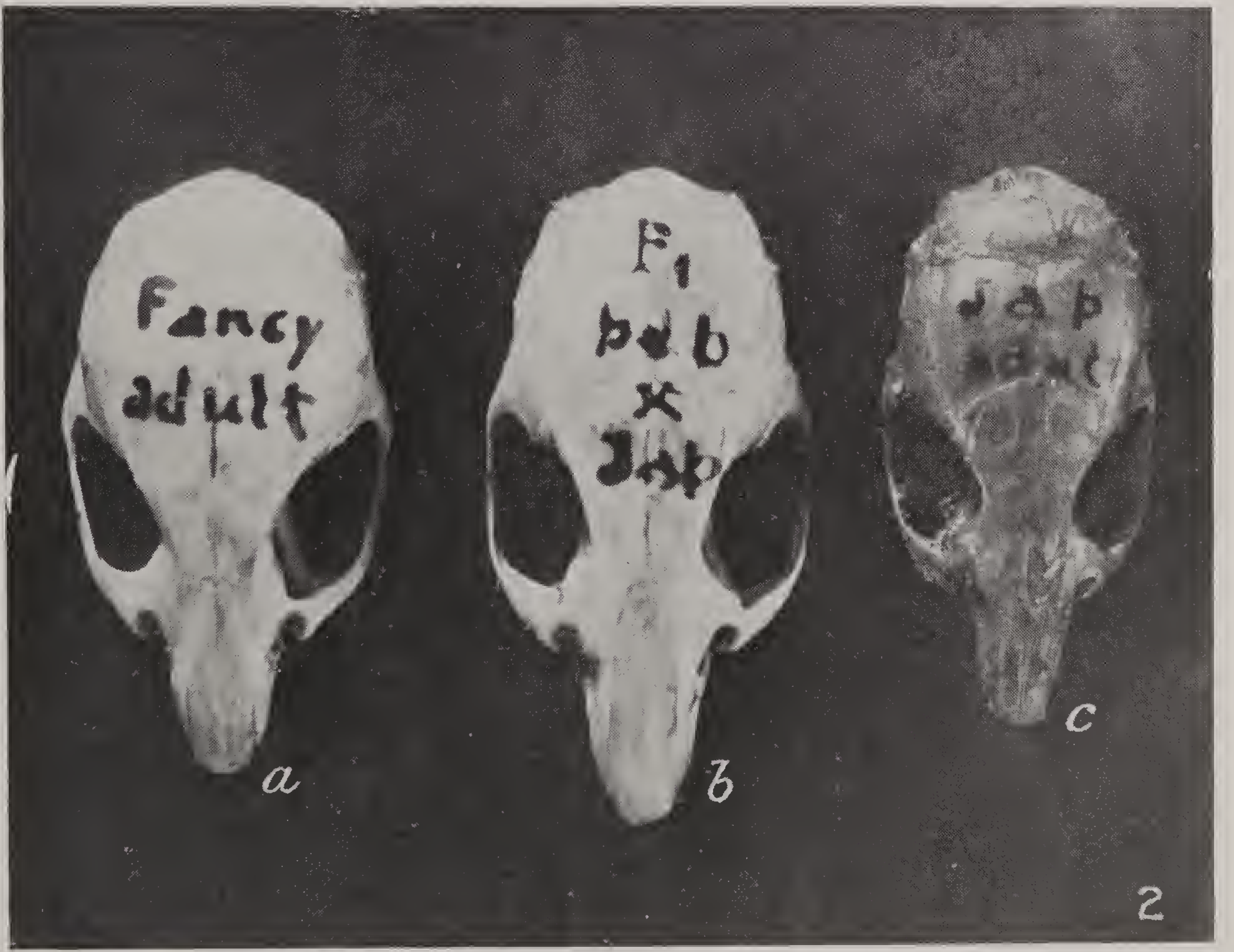

Fig. 2.—Skulls of adult (*a*) fancy mouse, (*c*) Japanese waltzer, and (*b*) F_1 hybrid between *a* and *c*, each selected for its representative character. 2.5 times natural size.

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